

Supplementary file to

Prognostic impact of expression of CD2, CD25, and/or CD30 in/on mast cells in systemic mastocytosis: a registry study of the ECNM

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BACKGROUND EUROPEAN COMPETENCE NETWORK ON MASTOCYTOSIS (ECNM)

The ECNM was established in 2002 as a non-profit network of an international group of scientists and clinicians working in the field of mastocytosis. As a collaborative initiative and scientific network of participating centers in Europe, it includes Reference Centers and Centers of Excellence [1,2]. There is a close collaboration of the members of the ECNM in order to discuss basic research as well as clinical concepts on diagnosis, prognostication and treatment of various forms of mast cell (MC) diseases with the aim to advance the field in this rare disease, involving specialists from different medical disciplines. With such an interdisciplinary approach connecting various medical fields and their specialists, including basic researchers, dermatologists, allergologists, hematologists or pathologists, all aspects of MC diseases are covered in the ECNM. Within the ECNM network, researchers and physicians take care of patients with various MC disorders, such as cutaneous and systemic mastocytosis (SM) as well as mast cell activation syndromes (MCAS). Moreover, genetic anomalies, such as hereditary alpha tryptasemia (H α T), are subject of research in ECNM projects. The members of the ECNM are organizing annual meetings with the aim of providing new information about MC diseases, including diagnostics and management strategies involving new therapeutic options for treatment of mastocytosis. These annual meetings are not only providing a platform for discussion and exchange of ideas and new developments in the field but are also offering new information for patient advocacy groups. Within the ECNM, active centers also promote education and the development of standards and optimal patient care and facilitate referral to medical experts and specialized centers in the field. A valuable source of all relevant information is the ECNM homepage at the Medical University of Vienna, Austria.

40 **THE ECNM REGISTRY**

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42 **Introduction**

43 The establishment of the ECNM registry has been an initiative of participating ECNM centers in
44 2012, aimed at creating a large set of data obtained from patients with various MC diseases. Patients
45 with cutaneous mastocytosis as well as patients with SM have been enrolled since 2012. SM patients
46 include cases with non-advanced forms, such as bone marrow mastocytosis (BMM), indolent SM
47 (ISM), or smoldering SM (SSM), and advanced forms, such as aggressive SM (ASM), SM with an
48 associated hematologic neoplasm (SM-AHN), and mast cell leukemia (MCL). Further aims of the
49 ECNM registry are to obtain data on the prevalence, etiology, diagnosis, prognosis and the clinical
50 course of all forms of MC diseases as well as additional potentially relevant disorders (co-morbidities)
51 [3].

52 To achieve these goals and evaluate data, several ECNM registry projects have been launched,
53 each of these projects focusing on a certain research topic.

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55 **Collecting Data in the ECNM Registry**

56 The ECNM registry is a web-based data base incorporating laboratory and clinical data of patients
57 with various MC diseases [3]. The majority of patients included in the ECNM registry are seen and
58 managed in the various Centers of Excellence of the ECNM. Data are collected both retro- and
59 prospectively and are entered in the ECNM registry either by the clinical research coordinator (CRC)
60 or the local principle investigator (PI) of such a center. Patient data are pseudonymized using a
61 unique code for each patient ensuring that identification of a patient is only possible for the CRC or
62 the PI of the local center. To ensure high quality data a regular data check is performed, there is a
63 data clearing once yearly, and technical adjustments are implemented as needed. Queries are sent
64 to the participating centers in case of inconsistencies or ambiguities. The Medical University of Vi-
65 enna, Austria, is the sponsor of the ECNM registry.

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67 **Participating Centers and ECNM Registry Projects**

68 There is an ECNM registry board consisting of the coordinators of the ECNM registry at the Medical
69 University of Vienna, Austria, and the local PI of the actively participating center. The ECNM registry
70 projects are based on a registry study and a related contract between the sponsor and the partic-
71 ipating centers where rights and obligations are defined. In fact, this consortium contract defines the
72 distribution, conduct, and development of ECNM registry projects in various participating centers.
73 Each actively participating center is invited to conduct one or more ECNM registry projects. The
74 contract also defines the role of the participating centers, collaborations within the ECNM registry
75 projects as well as details concerning publications. Participating centers are encouraged to conduct
76 one or more ECNM registry project and to publish the results.

80 **Inclusion and Exclusion Criteria in the ECNM Registry**

81 All patients with confirmed mastocytosis, based on the classification of the World Health Organiza-
82 tion (WHO), are potential candidates for inclusion in the ECNM registry [4,5]. This includes patients
83 with cutaneous mastocytosis, SM, and mast cell sarcoma. Patients in whom mastocytosis is not
84 confirmed or patients with mast cell activation syndromes (MCAS) without mastocytosis are ex-
85 cluded. There are no age restrictions to include patients in the ECNM registry. In other words, pedi-
86 atric and adult patients with confirmed mastocytosis at any age can be included. Patients with mast
87 cell infiltrates in the skin but no bone marrow examination, who have a provisional diagnosis of mas-
88 tocytosis in the skin (MIS), can also be included in the ECNM registry [6]. Patients with or without
89 allergies and patients with or without anaphylaxis can be included in the ECNM registry, provided
90 that they are suffering from confirmed mastocytosis.

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92 **Approval of Ethics Committee**

93 A written informed consent provided by the patient is a prerequisite for inclusion in the ECNM regis-
94 try. That means that all patients in the ECNM registry provided their written informed consent to be
95 included in the ECNM registry. The ECNM registry study was approved by the responsible local
96 ethics committee of the participating center.

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120 **SUPPLEMENTARY METHODS**121
122 **Determining associations between surface marker expression and disease-related parameters**
123124 Both, clinical and laboratory parameters, were analyzed with regard to a possible association with
125 the expression of CD2, CD25, and/or CD30 in clonal MC in our SM patients. Laboratory parameters
126 had to be comparable to analyze them in a statistical valuable approach. Laboratory values included
127 basal serum tryptase, *KIT* D816V mutation, other activating *KIT* mutations, as well as chromosomal
128 aberrations distinguishing between normal (46,XX and 46,XY) and abnormal (all other) karyotype.
129130 **Statistical analysis**131 Kaplan-Meier curves for overall survival, progression-free survival and event-free survival were con-
132 structed and stratified by expression patterns of CD2, CD25, and CD30 [7]. In the log-log transformed
133 survival curves of patients with CD2-positive MC und CD2-negative MC with indolent systemic mas-
134 tocytosis (ISM), smoldering systemic mastocytosis (SSM) and advanced systemic mastocytosis
135 (advSM) both curves do not cross, therefore the risk of having an event does not significantly change
136 over time.
137138 A log-rank test was employed to assess differences in survival time among ISM, SSM and advSM.
139140 P-values below 0.05 were considered to be statistically significant.
141142 The assumption of proportional hazards was tested by visual inspection of the log-log plots.
143144 Multivariate analyses were conducted using CD2 expression of MC, CD25 expression of MC, age at
145 inclusion and sex.
146147 Supplemental Table S1148 Variance inflation factors (VIF) in the variables CD2 expression of MC, CD25 expression of MC, age
149 and sex.
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Variable	Variance Inflation Factor (VIF)
CD2 expression of MC	1.08
CD25 expression of MC	1.09
Age at inclusion	1.01
Sex	1.0

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152 Multicollinearity was checked visually and by calculating the VIF listed in the table. There was no
153 evidence of multicollinearity (all VIF < 5).
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156 **SUPPLEMENTARY RESULTS**

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 158 **Overall survival (OS) in patients with SM stratified by CD2 expression pattern in three groups**
 159 **of MC disorders, indolent systemic mastocytosis (ISM), smoldering systemic mastocytosis**
 160 **(SSM) and advanced systemic mastocytosis (advSM), is depicted in the Supplementary Fig-**
 161 **ures S1 a, b, c.**

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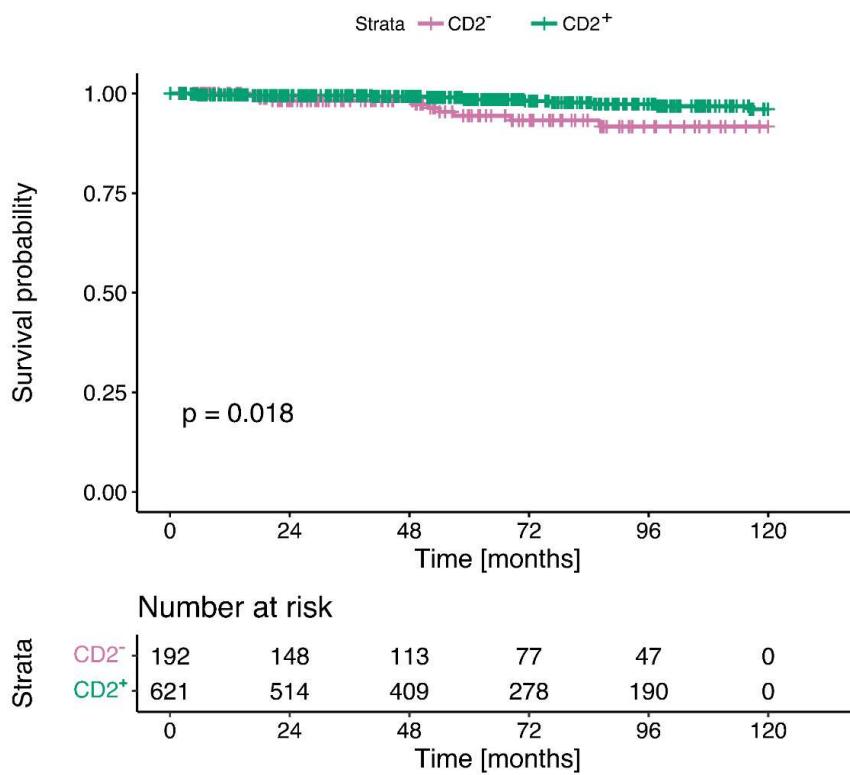
163 Supplemental Figures S1 a, b, c.

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165 Figure S1 a.

166 OS in patients with ISM stratified by CD2 expression pattern in MC

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170 OS was analyzed in a univariate analysis in patients with *indolent systemic mastocytosis* in whom
 171 FU and survival data were reported and results by flow cytometry and/or immunohistochemistry for
 172 CD2 on/in MC were available. Two groups of patients were examined based on the pattern of CD2
 173 expression on/in MC: CD2 negativity and CD2 positivity. The probability of OS in these two groups
 174 of patients was determined according to the method of Kaplan and Meier.

175 In patients with ISM there was a statistically significant reduction in OS with lack of expression of
 176 CD2 (p=0.018). The p-value refers to the comparison of survival curves as assessed by log-rank
 177 test.

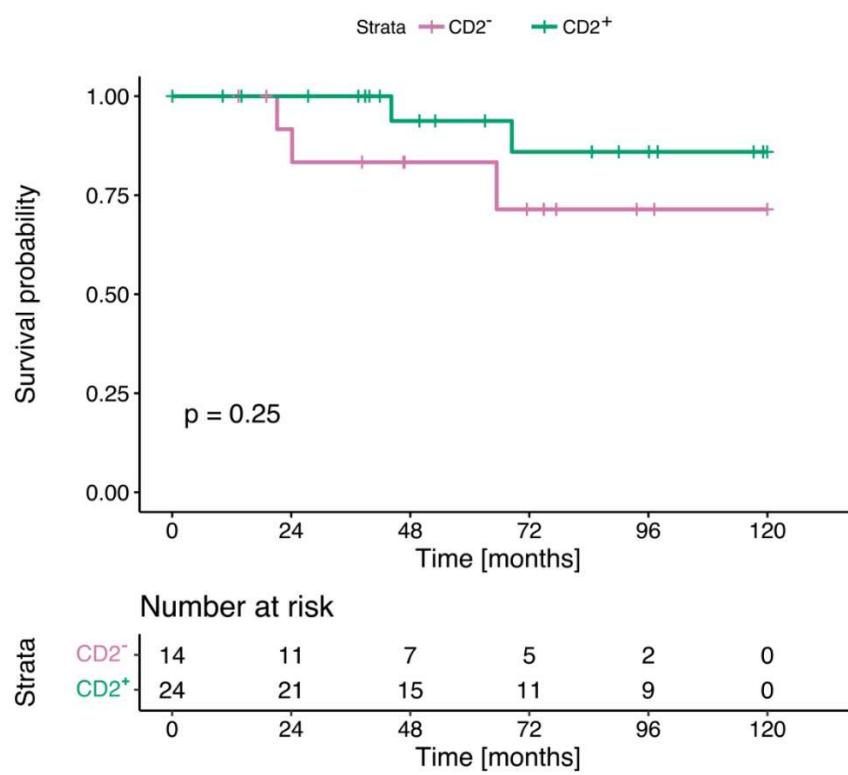
178 Abbreviations: OS, overall survival; ISM, indolent systemic mastocytosis, MC, mast cell; FU, follow-
 179 up.

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181 Figure S1 b.

182 OS in patients with SSM stratified by CD2 expression pattern in MC

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186 OS was analyzed in a univariate analysis in patients with *smoldering systemic mastocytosis* in whom
187 FU and survival data were reported and results by flow cytometry and/or immunohistochemistry for
188 CD2 on/in MC were available. Two groups of patients were examined based on the pattern of CD2
189 expression on/in MC: CD2 negativity and CD2 positivity. The probability of OS in these two groups
190 of patients was determined according to the method of Kaplan and Meier.

191 In patients with SSM there was a trend towards a reduced OS in patients with lack of expression of
192 CD2 (p=0.25). The p-value refers to the comparison of survival curves as assessed by log-rank test.
193 Abbreviations: OS, overall survival; SSM, smoldering systemic mastocytosis, MC, mast cell; FU,
194 follow-up.

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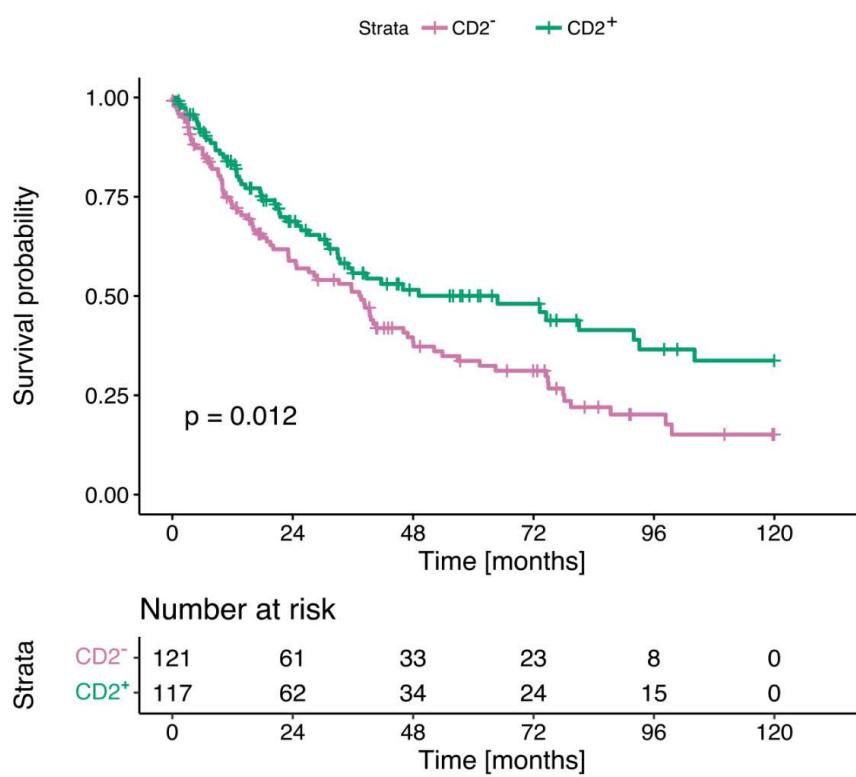
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206 Figure S1 c.

207 OS in patients with advSM stratified by CD2 expression pattern in MC

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211 OS was analyzed in a univariate analysis in patients with *advanced systemic mastocytosis* in whom
212 FU and survival data were reported and results by flow cytometry and/or immunohistochemistry for
213 CD2 on/in MC were available. Two groups of patients were examined based on the pattern of CD2
214 expression on/in MC: CD2 negativity and CD2 positivity. The probability of OS in these two groups
215 of patients was determined according to the method of Kaplan and Meier.

216 In patients with advSM there was a statistically significant reduction in OS with lack of expression of
217 CD2 ($p=0.012$). The p-value refers to the comparison of survival curves as assessed by log-rank
218 test.

219 Abbreviations: OS, overall survival; advSM, advanced systemic mastocytosis, MC, mast cell; FU,
220 follow-up.

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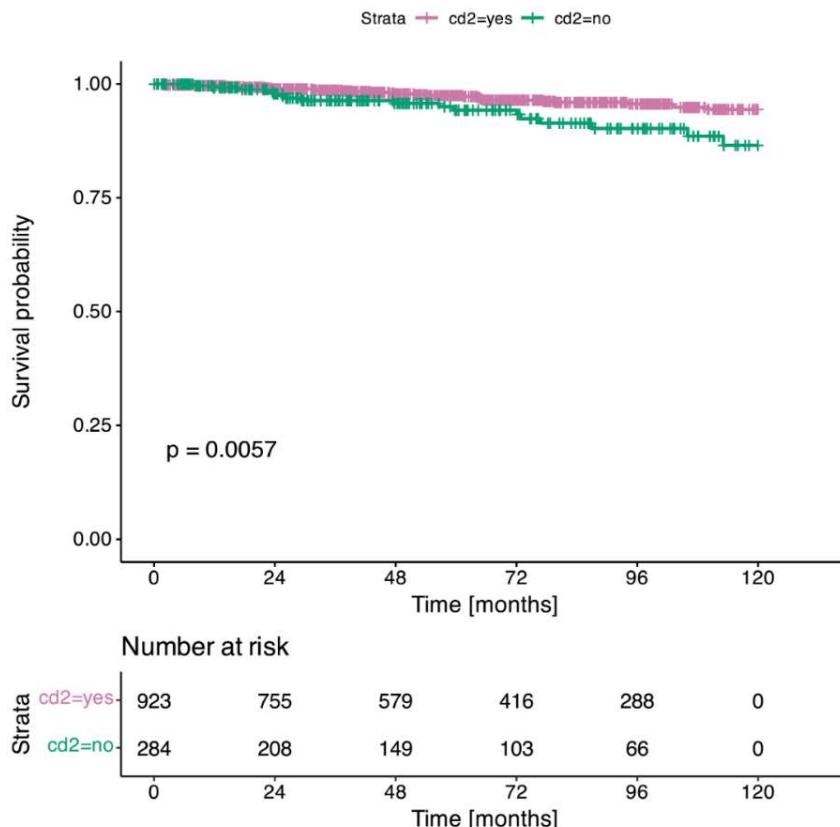
231 **Progression-free survival (PFS) in patients with ISM and with advSM stratified by CD2 expres-**
232 **sion pattern in MC, and in patients with SM stratified by CD2 and CD25 expression patterns**
233 **in MC, is depicted in the Supplementary Figures S2 to S4.**

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235 Supplemental Figure S2

236 PFS in patients with ISM stratified by CD2 expression pattern in MC

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240 PFS was analyzed in a univariate analysis in patients with *indolent systemic mastocytosis* in whom
241 FU and survival data were reported and results by flow cytometry and/or immunohistochemistry for
242 CD2 on/in MC were available. Two groups of patients were examined based on the pattern of CD2
243 expression on/in MC: CD2 negativity and CD2 positivity. The probability of PFS in these two groups
244 of patients was determined according to the method of Kaplan and Meier.

245 PFS in patients with ISM is influenced by CD2 expression. There is a significantly reduced PFS in
246 patients with ISM and lack of CD2 expression ($p=0.0057$). The p-value refers to the comparison of
247 survival curves as assessed by log-rank test.

Abbreviations: PFS, progression-free survival; ISM, indolent systemic mastocytosis; MC, mast cell; FU, follow-up.

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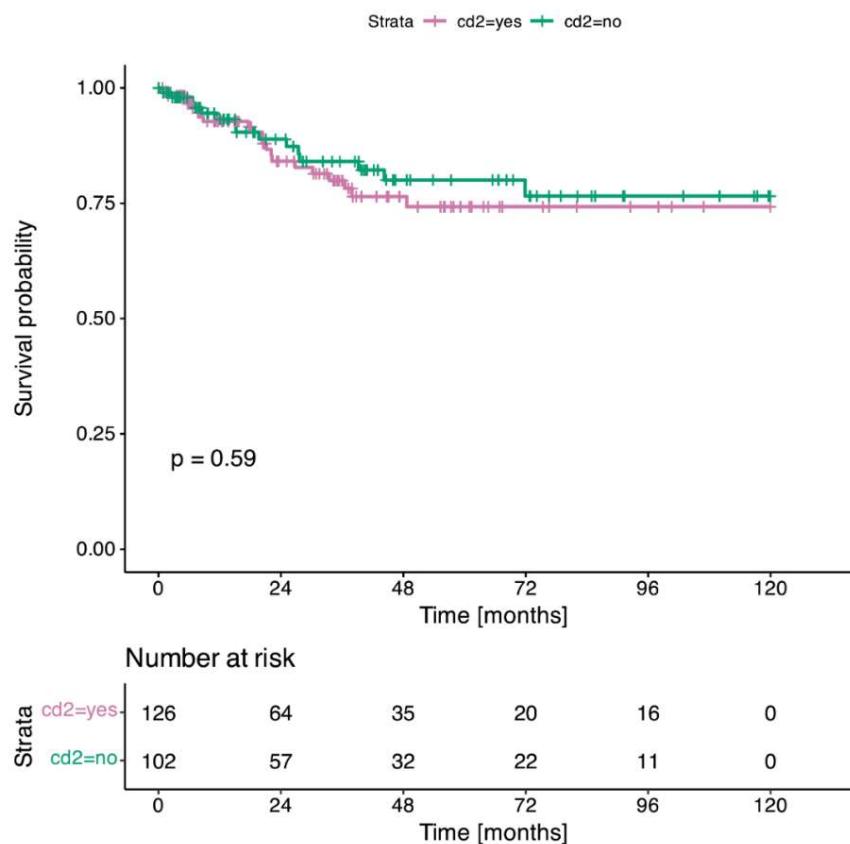
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255 Supplemental Figure S3

256 PFS in patients with advSM stratified by CD2 expression pattern in MC

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260 PFS was analyzed in a univariate analysis in patients with *advanced systemic mastocytosis* in whom
261 FU and survival data were reported and results by flow cytometry and/or immunohistochemistry for
262 CD2 on/in MC were available. Two groups of patients were examined based on the pattern of CD2
263 expression on/in MC: CD2 negativity and CD2 positivity. The probability of PFS in these two groups
264 of patients was determined according to the method of Kaplan and Meier.

265 PFS in patients with advSM is not influenced by CD2 expression (p=0.59). The p-value refers to the
266 comparison of survival curves as assessed by log-rank test.

267 Abbreviations: PFS, progression-free survival; advSM, advanced systemic mastocytosis; MC, mast
268 cell; FU, follow-up.

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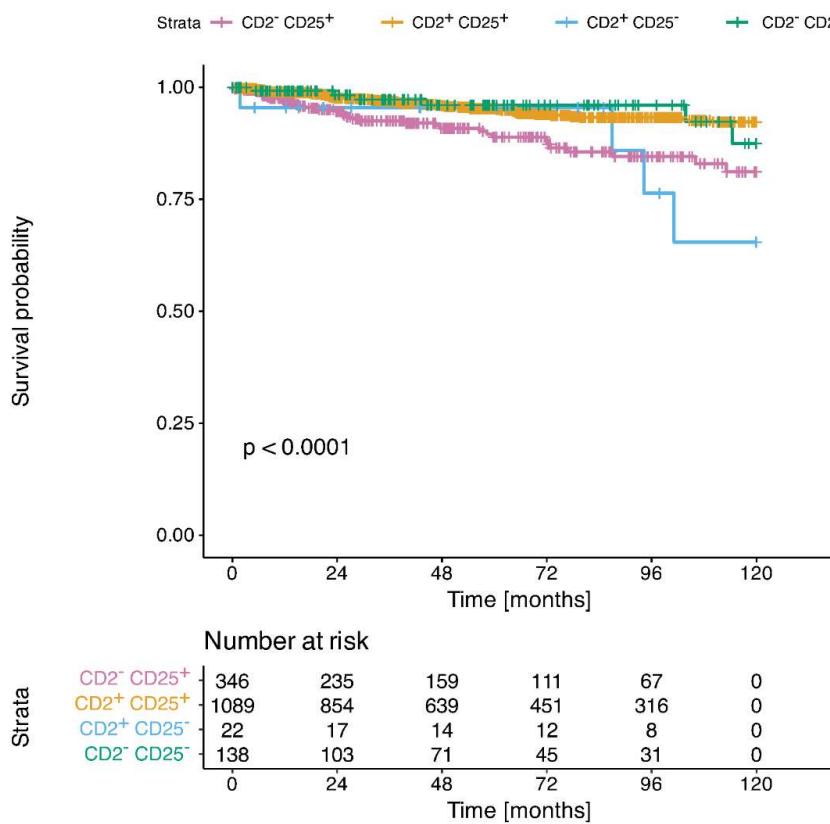
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278 Supplemental Figure S4

279 PFS in patients with SM stratified by CD2 and CD25 expression patterns in MC

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284 PFS was analyzed in a univariate analysis in 1183 patients with *systemic mastocytosis* in whom FU
 285 and survival data were reported and results by flow cytometry and/or immunohistochemistry for CD2
 286 expression and CD25 expression on/in MC were available. Four groups of patients were examined
 287 based on the pattern of CD2 expression and CD25 expression on/in MC: CD2-/CD25+ MC,
 288 CD2+/CD25+ MC, CD2+/CD25- MC; and CD2-/CD25- MC. The probability of PFS in these 4 groups
 289 of patients was determined according to the method of Kaplan and Meier.

290 Patients with CD2-negative MC expressing CD25 had a significantly reduced PFS compared to pa-
 291 tients with MC expressing both, CD2 and CD25 ($p < 0.0001$). The p-value refers to the comparison of
 292 all survival curves as assessed by log-rank test.

293 Abbreviations: PFS, progression-free survival; SM, systemic mastocytosis; MC, mast cell; FU, fol-
 294 low-up.

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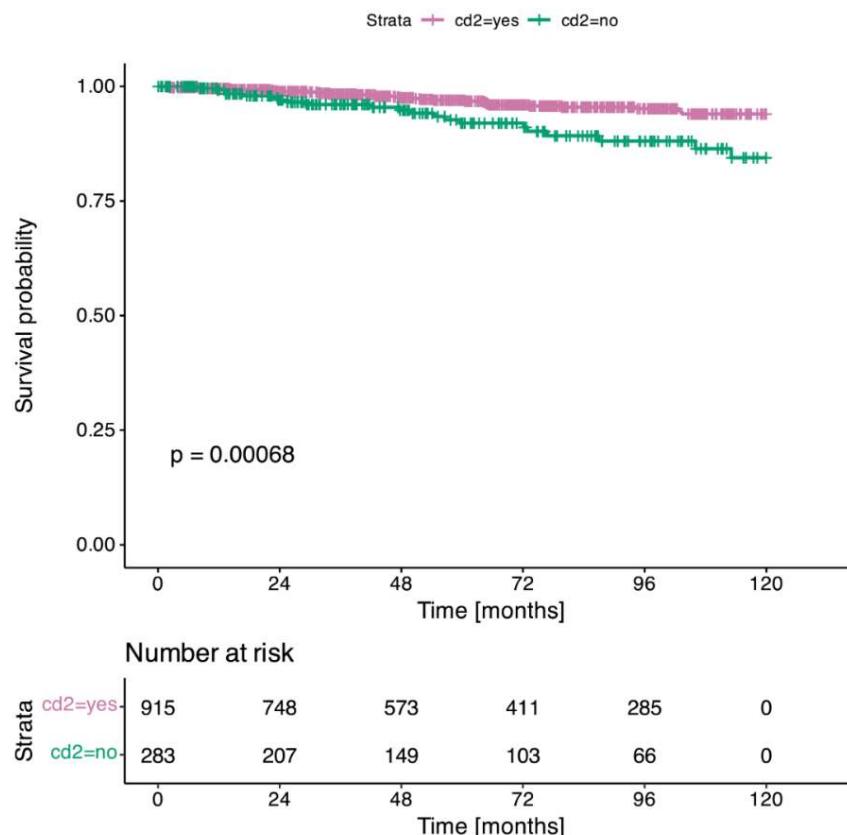
302 **Event-free survival (EFS) in patients with ISM and with advSM stratified by CD2 expression**
303 **pattern in MC, and in patients with SM stratified by CD2 and CD25 expression patterns in MC,**
304 **is depicted in the Supplementary Figures S5 to S7.**

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306 Supplemental Figure S5

307 EFS in patients with ISM stratified by CD2 expression pattern in MC

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311 EFS was analyzed in a univariate analysis in patients with *indolent systemic mastocytosis* in whom
312 FU and survival data were reported and results by flow cytometry and/or immunohistochemistry for
313 CD2 on/in MC were available. Two groups of patients were examined based on the pattern of CD2
314 expression on/in MC: CD2 negativity and CD2 positivity. The probability of EFS in these two groups
315 of patients was determined according to the method of Kaplan and Meier.

316 EFS in patients with ISM is influenced by CD2 expression. There is a significantly reduced EFS in
317 patients with ISM and lack of CD2 expression ($p=0.00068$). The p-value refers to the comparison of
318 survival curves as assessed by log-rank test.

319 Abbreviations: EFS, event-free survival; ISM, indolent systemic mastocytosis; MC, mast cell; FU,
320 follow-up.

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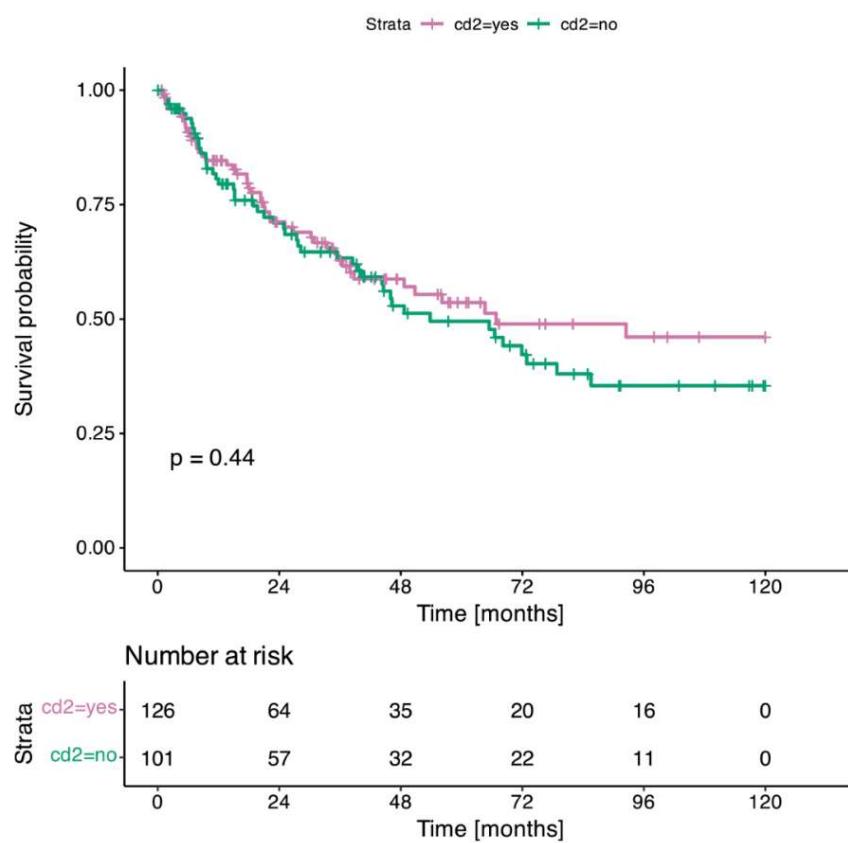
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326 Supplemental Figure S6

327 EFS in patients with advSM stratified by CD2 expression pattern in MC

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331 EFS was analyzed in a univariate analysis in patients with *advanced systemic mastocytosis* in whom
 332 FU and survival data were reported and results by flow cytometry and/or immunohistochemistry for
 333 CD2 on/in MC were available. Two groups of patients were examined based on the pattern of CD2
 334 expression on/in MC: CD2 negativity and CD2 positivity. The probability of EFS in these two groups
 335 of patients was determined according to the method of Kaplan and Meier.

336 EFS in patients with advSM is not influenced by CD2 expression ($p=0.44$). The p-value refers to the
 337 comparison of survival curves as assessed by log-rank test.

338 Abbreviations: EFS, event-free survival; advSM, advanced systemic mastocytosis; MC, mast cell;
 339 FU, follow-up.

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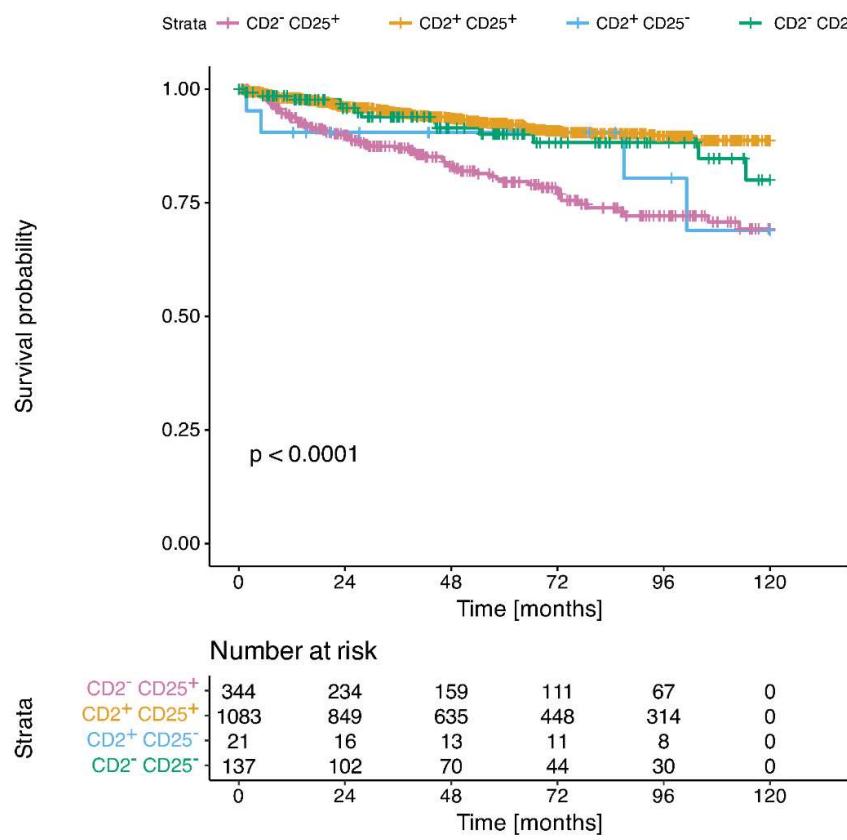
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350 Supplemental Figure S7

351 EFS in patients with SM stratified by CD2 and CD25 expression patterns in MC

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356 EFS was analyzed in a univariate analysis in 1183 patients with *systemic mastocytosis* in whom FU
 357 and survival data were reported and results by flow cytometry and/or immunohistochemistry for CD2
 358 expression and CD25 expression on/in MC were available. Four groups of patients were examined
 359 based on the pattern of CD2 and CD25 expression on/in MC: CD2-/CD25+ MC, CD2+/CD25+ MC,
 360 CD2+/CD25- MC; and CD2-/CD25- MC. The probability of EFS in these 4 groups of patients was
 361 determined according to the method of Kaplan and Meier.

362 Patients with CD2-negative MC expressing CD25 had a significantly reduced EFS compared to pa-
 363 tients with MC expressing both, CD2 and CD25 (p<0.0001). The p-value refers to the comparison of
 364 all survival curves as assessed by log-rank test.

365 Abbreviations: EFS, event-free survival; SM, systemic mastocytosis; MC, mast cell; FU, follow-up.

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374 Due to the small number of patients with SSM, the majority having CD25 positive MC, establishment
375 of contrast for CD25 was not done.

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377 **Survival analysis with multivariate analysis in patients with SM, taking into account CD2 ex-**
378 **pression and CD25 expression, age and sex, are summarized in the Supplementary Tables**
379 **S2 to S4.**

380

381 **Supplementary Table S2**

382 Multivariate analysis of *overall survival* in patients with SM

383

Variable	Overall			ISM			SSM			AdvSM		
	HR ¹	95% CI ²	p-value	HR ¹	95% CI ²	p-value	HR ¹	95% CI ²	p-value	HR ¹	95% CI ²	p-value
CD2 ⁺ MC ³	-	-		-	-		-	-		-	-	
CD2 ⁻ MC ³	2.73	1.97, 3.78	< 0.001	2.73	1.12, 6.67	0.027	0.83	0.10, 6.61	0.9	1.41	1.0, 2.01	0.053
CD25 ⁺ MC ³	-	-		-	-		-	-		-	-	
CD25 ⁺ MC ³	1.53	0.85, 2.75	0.2	1.63	0.2, 13.0	0.6				0.77	0.39, 1.53	0.5
Age	1.09	1.08, 1.11	< 0.001	1.14	1.09, 1.20	< 0.001	1.30	1.04, 1.63	0.023	1.04	1.02, 1.05	< 0.001
Female	-	-		-	-		-	-		-	-	
Male	1.95	1.4, 2.71	< 0.001	1.13	0.47, 2.69	0.8	1.8	0.23, 14.3	0.6	1.36	0.94, 1.99	0.11

384 ¹HR, Hazard Ratio; ²CI, Confidence Interval; ³MC, mast cell

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386 OS calculation with multivariate analysis in indolent systemic mastocytosis (ISM), smoldering sys-
387 temic mastocytosis (SSM) and advanced systemic mastocytosis (advSM), taking into account CD2
388 expression and CD25 expression in MC, age and sex.

389 There is a significantly reduced OS in patients with SM with CD2-negative MC in the whole cohort
390 and in patients with ISM.

391

392 **Supplementary Table S3**

393 Multivariate analysis of *progression-free survival* in patients with SM

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Variable	Overall			ISM			SSM			AdvSM		
	HR ¹	95% CI ²	p-value	HR ¹	95% CI ²	p-value	HR ¹	95% CI ²	p-value	HR ¹	95% CI ²	p-value
CD2 ⁺ MC ³	-	-		-	-		-	-		-	-	
CD2 ⁻ MC ³	2.18	1.40, 3.40	< 0.001	2.49	1.34, 4.60	0.004	0.83	0.10, 6.61	0.9	0.7	0.34, 1.42	0.3
CD25 ⁺ MC ³	-	-		-	-		-	-		-	-	
CD25 ⁺ MC ³	1.2	0.60, 2.44	0.6	1.85	0.43, 7.96	0.4				0.89	0.21, 3.83	0.9
Age	1.02	1.00, 1.04	0.011	1.00	0.98, 1.02	> 0.9	1.3	1.04, 1.63	0.023	1.02	0.99, 1.04	0.2
Female	-	-		-	-		-	-		-	-	
Male	1.53	1.01, 2.32	0.043	1.25	0.7, 2.23	0.5	1.8	0.23, 14.3	0.6	1.97	0.91, 4.26	0.084

395 ¹HR, Hazard Ratio; ²CI, Confidence Interval; ³MC, mast cell

396

397 PFS calculation with multivariate analysis in indolent systemic mastocytosis (ISM), smoldering sys-
398 temic mastocytosis (SSM) and advanced systemic mastocytosis (advSM), taking into account CD2
399 expression und CD25 expression in MC, age and sex.

400 There is a significantly reduced PFS in patients with SM with CD2-negative MC in the whole cohort
401 and in patients with ISM.

402

403 Supplementary Table S4

404 Multivariate analysis of *event-free survival* in patients with SM

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Variable	Overall			ISM			SSM			AdvSM		
	HR ¹	95% CI ²	p-value	HR ¹	95% CI ²	p-value	HR ¹	95% CI ²	p-value	HR ¹	95% CI ²	p-value
CD2 ⁺ MC ³	-	-		-	-		-	-		-	-	
CD2 ⁻ MC ³	2.40	1.73, 3.33	< 0.001	2.81	1.60, 4.93	< 0.001	0.83	0.10, 6.61	0.9	1.00	0.66, 1.53	> 0.9
CD25 ⁺ MC ³	-	-		-	-		-	-		-	-	
CD25 ⁻ MC ³	1.23	0.72, 2.09	0.4	2.09	0.49, 8.89	0.3				0.7	0.33, 1.47	0.3
Age	1.05	1.04, 1.06	< 0.001	1.03	1.01, 1.05	0.009	1.3	1.04, 1.63	0.023	1.03	1.01, 1.05	< 0.001
Female	-	-		-	-		-	-		-	-	
Male	1.63	1.19, 2.22	0.002	1.09	0.63, 1.87	0.8	1.8	0.23, 14.3	0.6	1.68	1.07, 2.64	0.023

¹ HR, Hazard Ratio; ² CI, Confidence Interval; ³ MC, mast cell

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408 EFS calculation with multivariate analysis in indolent systemic mastocytosis (ISM), smoldering sys-
409 temic mastocytosis (SSM) and advanced systemic mastocytosis (advSM), taking into account CD2
410 expression und CD25 expression in MC, age and sex.

411 There is a significantly reduced EFS in patients with SM with CD2-negative MC in the whole cohort
412 and in patients with ISM.

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433 **Reasons of death in patients with SM are depicted in the Supplemental Table S5.**

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435 **Supplementary Table S5**

436 Reasons of death in patients with SM

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Variable	ISM	SSM	AdvSM	p-value	Missing values (n %)
Death	32 (2.3)	6 (10.7)	212 (53.5)	<0.001	735 (25.9)
Reasons of death (%)				<0.001	5 (2.0)
Disease related	7 (25.0)	2 (33.3)	140 (66.0)	<0.01	
Other	17 (53.1)	4 (66.7)	38 (18.4)	<0.01	
Treatment related	1 (3.1)	0 (0.0)	13 (6.1)	0.65	
Unknown	5 (15.6)	0 (0.0)	18 (8.9)	0.35	

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439 Analyzing reasons of death and comparing indolent systemic mastocytosis (ISM), smoldering sys-
440 temic mastocytosis (SSM) and advanced systemic mastocytosis (advSM), death was significantly
441 less disease related in ISM compared to SSM and advSM, although power of statistical analysis is
442 limited due to small number of cases. In two patients with ISM and in three patients with advSM there
443 were missing values.

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468 **Associations between expression of aberrant CD2 and CD25 in MC with other clinical and**
 469 **laboratory parameters in multivariate analysis are summarized in the Supplementary Tables**
 470 **S6 to S8.**

471

472 Supplemental Table S6

473 Multivariate analysis of allergies, constitutional / cardiovascular symptoms and SM-related osteopa-
 474 thy in patients with SM

475

Variable	Allergy			Constitutional / Cardiovascular symptoms			SM-related osteopathy (osteopenia and osteoporosis)		
	OR ¹	95% CI ²	p-value	OR ¹	95% CI ²	p-value	OR ¹	95% CI ²	p-value
CD2 ⁺ MC ³	-	-		-	-		-	-	
CD2 ⁻ MC ³	0.53	0.42, 0.67	< 0.001	0.65	0.50, 0.84	< 0.001	0.62	0.49, 0.79	<0.001
CD25 ⁻ MC ³	-	-		-	-		-	-	
CD25 ⁺ MC ³	1.37	0.88, 2.17	0.200	1.44	0.88, 2.45	0.3	1.21	0.78, 1.93	0.4
Age	1.00	0.99, 1.00	0.120	0.99, 1.00	1.01, 1.05	0.009	1.03	1.02, 1.03	<0.001
Female	-	-		-	-		-	-	
Male	1.54	1.27, 1.87	< 0.001	0.96	0.78, 1.17	0.8	0.91	0.75, 1.12	0.4

476 ¹ OR, Odds Ratio; ² CI, Confidence Interval; ³ MC, mast cell

477

478 Multivariate analysis of allergies, constitutional / cardiovascular symptoms and SM-related osteopa-
 479 thy (osteopenia and osteoporosis), adjusted for sex and age.

480 CD2-negativity was associated with lower rates of allergies, constitutional / cardiovascular symptoms
 481 and SM-related osteopathy.

482

483 Supplemental Table S7

484 Multivariate analysis of pruritus, blistering/bullae and gastrointestinal (GI) symptoms in patients with
 485 SM

486

Variable	Pruritus (≥ mild or moderate frequent)			Blistering/Bullae			GI symptoms		
	OR ¹	95% CI ²	p-value	OR ¹	95% CI ²	p-value	OR ¹	95% CI ²	p-value
CD2 ⁺ MC ³	-	-		-	-		-	-	
CD2 ⁻ MC ³	0.76	0.56, 1.03	0.082	1.06	0.54, 1.95	0.9	1.09	0.87, 1.36	0.5
CD25 ⁻ MC ³	-	-		-	-		-	-	
CD25 ⁺ MC ³	0.99	0.59, 1.71	>0.9	1.08	0.39, 3.86	0.9	1.17	0.77, 1.77	0.5
Age	1.01	1.00, 1.02	0.064	1.00	0.98, 1.02	>0.9	0.99	0.99, 1.00	0.040
Female	-	-		-	-		-	-	
Male	0.87	0.68, 1.12	0.3	1.48	0.87, 2.50	0.14	0.49	0.41, 0.59	<0.001

487 ¹ OR, Odds Ratio; ² CI, Confidence Interval; ³ MC, mast cell

488

489 Multivariate analysis of pruritus, blistering/bullae and GI symptoms, adjusted for sex and age.

490 CD2-negativity was not associated with the presence of pruritus, blistering/bullae or GI symptoms.

491

492

493 Supplemental Table S8494 Multivariate analysis of karyotype and *KIT* mutations in patients with SM

495

	CD2 ⁻		CD25 ⁺		CD2 ⁻ CD25 ⁺	
	OR ¹ [95% CI ²]	p-value	OR ¹ [95% CI ²]	p-value	OR ¹ [95% CI ²]	p-value
Abnormal karyotype	1.32 [0.69, 2.5]	0.4	0.69 [0.28, 2.11]	0.5	1.11 [0.57, 2.13]	0.8
<i>KIT</i> mutations						
c- <i>KIT</i> D816V	0.36 [0.27, 0.47]	< 0.001	5.76 [3.85, 8.55]	< 0.001	0.63 [0.48, 0.88]	0.005
Other <i>KIT</i> -mutations	0.38 [0.10, 1.14]	0.1	3.86 [0.77, 70.3]	0.2	0.57 [0.12, 1.92]	0.4
Mutations in genes other than <i>KIT</i>	7.54 [1.90, 50.6]	0.011	0.35 [0.01, 3.94]	0.5	5.43 [1.36, 36.6]	0.065

496 ¹ OR, Odds Ratio; ² CI, Confidence Interval

497

498 Multivariate analysis of karyotype and *KIT* mutations, adjusted for sex and age, in patients with SM.
 499 There was a positive association between CD2-negativity and an abnormal karyotype with conventional cytogenetic analysis, which did not reach statistical significance. However, CD2-negativity was
 500 associated with mutations in genes other than *KIT* with an odds ratio of 7.54.

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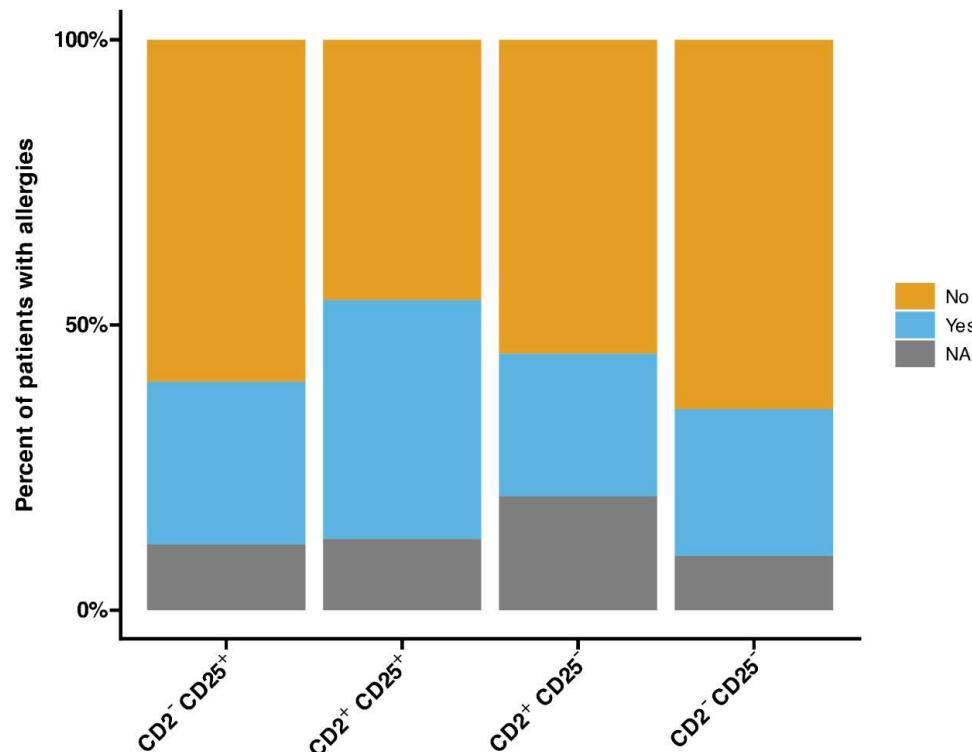
527 **Associations between expression of CD2 and CD25 in MC and selected *clinical* and *laboratory***
528 **parameters are depicted in Figures S8 to S13.**

529

530 Supplemental Figure S8

531 Percentage of patients with SM and *allergies* stratified by CD2 and CD25 expression patterns in MC

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534

535 Four groups of patients with SM and allergies were examined based on the pattern of CD2 and CD25
536 expression on/in MC as determined by flow cytometry or immunohistochemistry: CD2-/CD25+ MC,
537 CD2+/CD25+ MC, CD2+/CD25- MC; and CD2-/CD25- MC. CD2-negativity was associated with lower
538 rates of allergies (OR: 0.53; 95% CI 0.42, 0.67; $p < 0.001$).

539 Abbreviations: SM, systemic mastocytosis; MC, mast cell; NA, not applicable (no data available).

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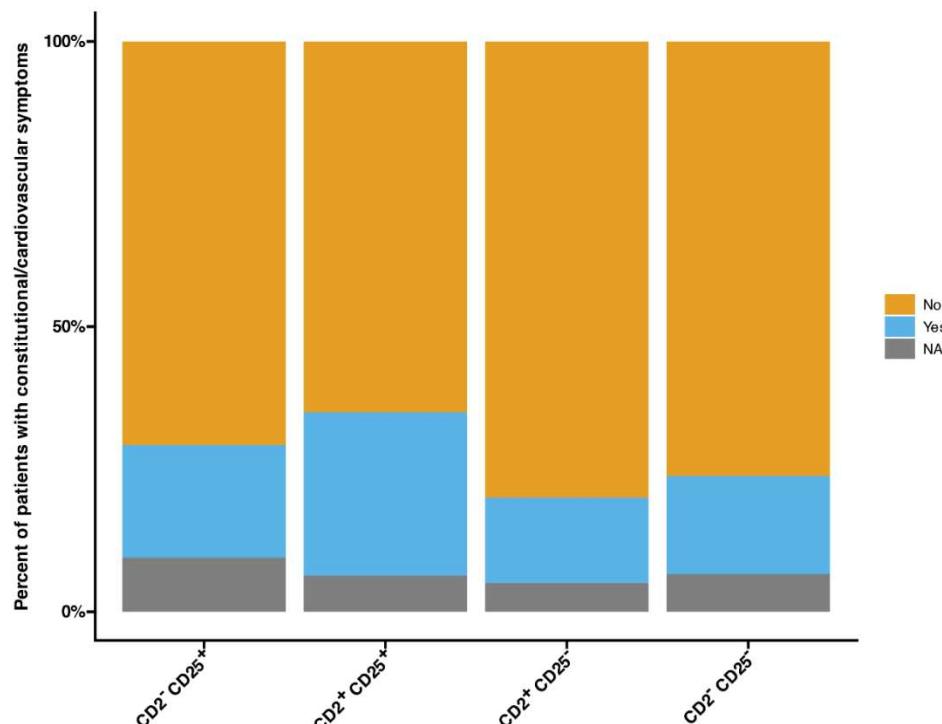
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552 Supplemental Figure S9

553 Percentage of patients with SM and *constitutional/cardiovascular symptoms* stratified by CD2 and
554 CD25 expression patterns in MC

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558 Four groups of patients with SM and constitutional/cardiovascular symptoms were examined based
559 on the pattern of CD2 and CD25 expression on/in MC as determined by flow cytometry or immuno-
560 histochemistry: CD2-/CD25⁺ MC, CD2⁺/CD25⁺ MC, CD2⁺/CD25⁻ MC; and CD2-/CD25⁻ MC. CD2-
561 negativity was associated with lower rates of constitutional/cardiovascular symptoms (OR: 0.65; 95%
562 CI: 0.50, 0.84; p=0.001).

563 Abbreviations: SM, systemic mastocytosis; MC, mast cell; NA, not applicable (no data available).

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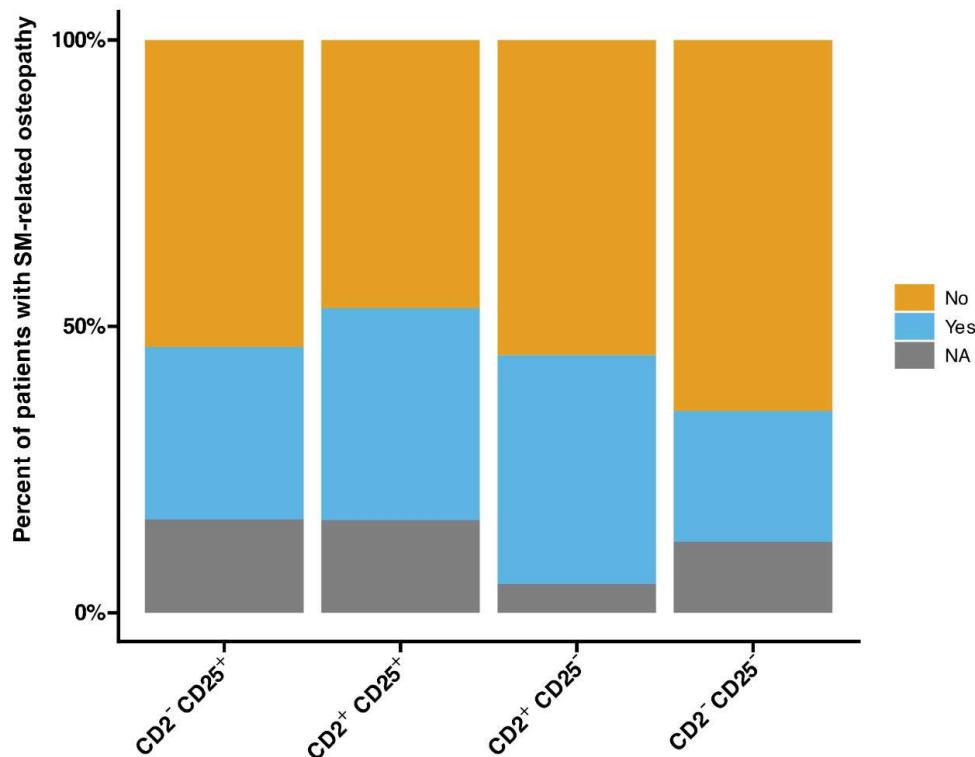
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578 Supplemental Figure S10579 Percentage of patients with SM and *SM-related osteopathy* (osteopenia or osteoporosis) stratified
580 by CD2 and CD25 expression patterns in MC

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584 Four groups of patients with SM and SM-related osteopathy (osteopenia or osteoporosis) were ex-
585 amined based on the pattern of CD2 and CD25 expression on/in MC as determined by flow cytom-
586 etry or immunohistochemistry: CD2-/CD25⁺ MC, CD2⁺/CD25⁺ MC, CD2⁺/CD25⁻ MC; and CD2⁻
587 /CD25⁻ MC. CD2-negativity was associated with lower rates of SM-related osteopathy with osteo-
588 penia or osteoporosis (OR: 0.62; 95% CI: 0.49, 0.79; p < 0.001).

589 Abbreviations: SM, systemic mastocytosis; MC, mast cell; NA, not applicable (no data available).

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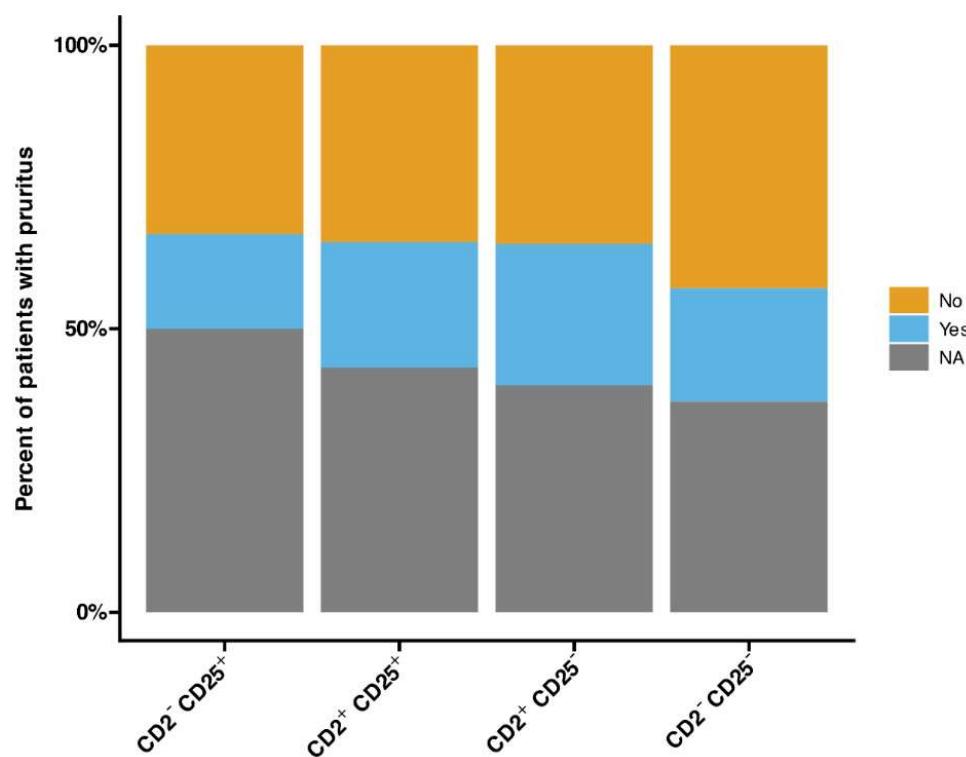
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603 Supplemental Figure S11604 Percentage of patients with SM and *pruritus* stratified by CD2 and CD25 expression patterns in MC

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608 Four groups of patients with SM and pruritus were examined based on the pattern of CD2 and CD25
609 expression on/in MC as determined by flow cytometry or immunohistochemistry: CD2-/CD25⁺ MC,
610 CD2⁺/CD25⁺ MC, CD2⁺/CD25⁻ MC; and CD2-/CD25⁻ MC. No difference was detected between the
611 severity of pruritus and different antigen marker expression.

612 Abbreviations: SM, systemic mastocytosis; MC, mast cell; NA, not applicable (no data available).

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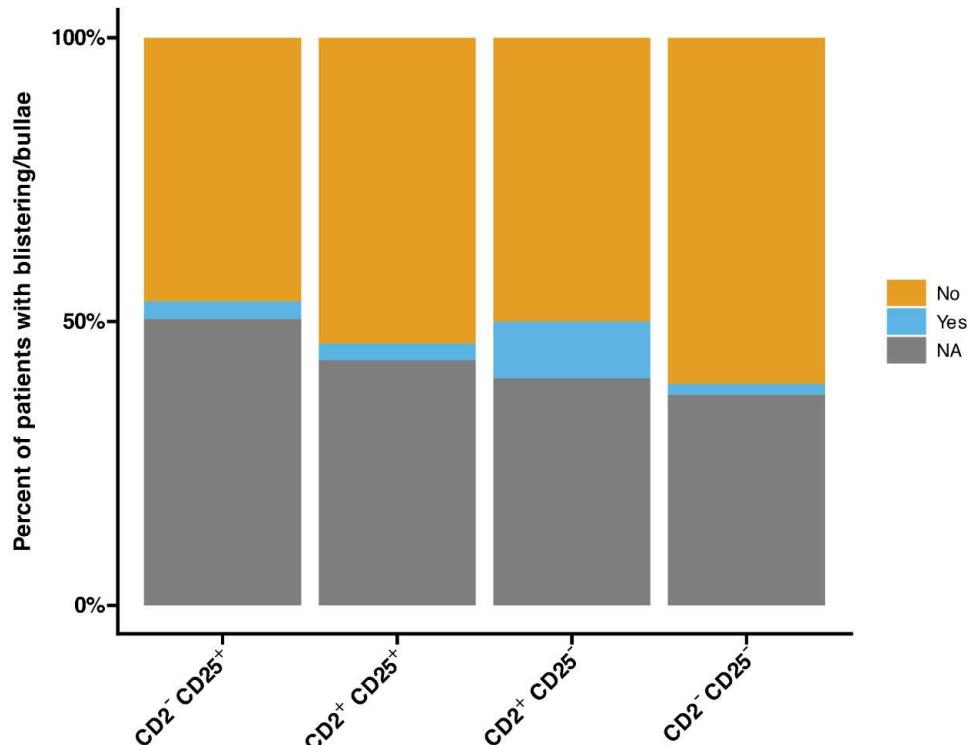
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628 Supplemental Figure S12

629 Percentage of patients with SM and *blistering or bullae* stratified by CD2 and CD25 expression pat-
630 terns in MC

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634 Four groups of patients with SM and blistering or bullae were examined based on the pattern of CD2
635 and CD25 expression on/in MC as determined by flow cytometry or immunohistochemistry: CD2-
636 /CD25⁺ MC, CD2⁺/CD25⁺ MC, CD2⁺/CD25⁻ MC; and CD2⁻/CD25⁻ MC. No difference was detected
637 between the severity of blistering of bullae and different antigen marker expression.

638 Abbreviations: SM, systemic mastocytosis; MC, mast cell; NA, not applicable (no data available).

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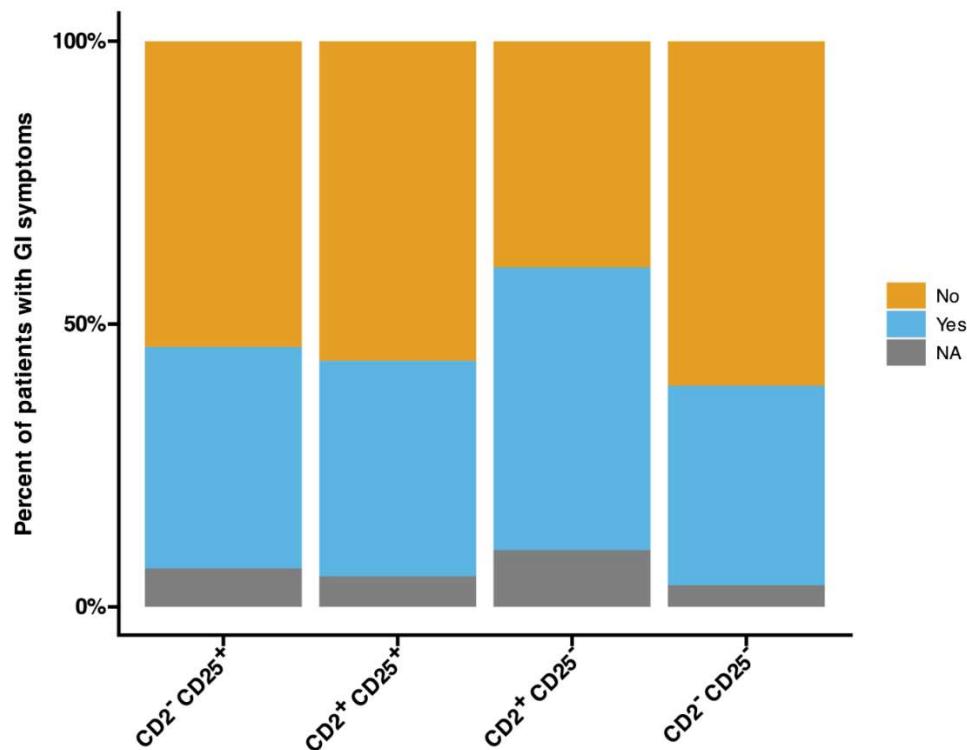
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653 Supplemental Figure S13

654 Percentage of patients with SM and *gastrointestinal symptoms* stratified by CD2 and CD25 expres-
655 sion patterns in MC

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659 Four groups of patients with SM and GI symptoms were examined based on the pattern of CD2 and
660 CD25 expression on/in MC as determined by flow cytometry or immunohistochemistry: CD2-/CD25+
661 MC, CD2+/CD25+ MC, CD2+/CD25- MC; and CD2-/CD25- MC. No difference was detected between
662 the absence of presence of GI symptoms and different antigen marker expression.

663 Abbreviations: SM, systemic mastocytosis; MC, mast cell; GI, gastrointestinal; NA, not applicable
664 (no data available).

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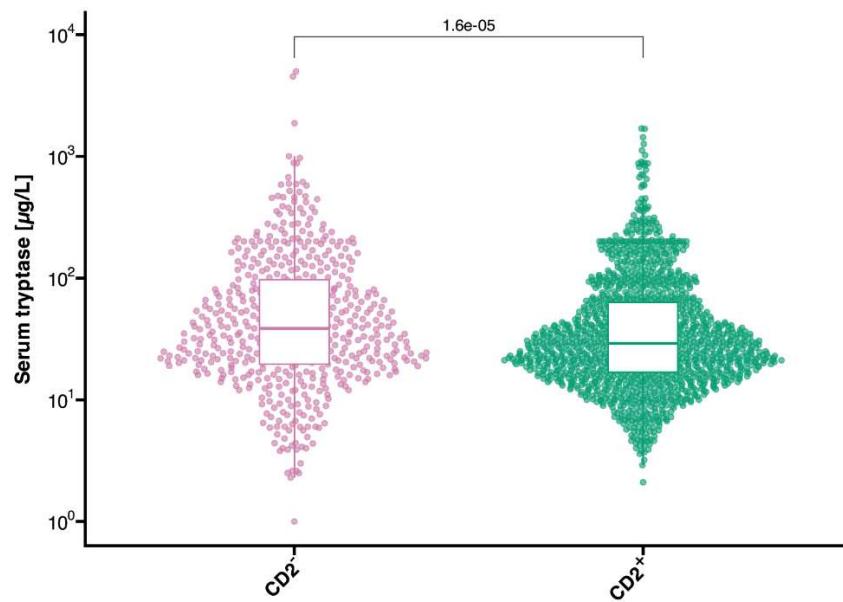
678 **Associations between serum tryptase level and expression of CD2 and CD25 in MC are de-**
679 **picted in Figures S14 to S15.**

680

681 Supplemental Figure S14

682 Serum tryptase level in patients with SM stratified by CD2 expression pattern in MC

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686 Basal serum tryptase (BST) level was analyzed in 531 patients with SM with CD2 negative MC and
687 in 1441 patients with SM with CD2 positive MC.

688 The median BST was 40.55 µg/l (IQR: 21.00, 102.00) and 29.00 µg/l (IQR: 17.00, 63.40), in CD2-
689 negative MC and in CD2-positive MC, respectively ($p < 0.001$).

690 Abbreviations: SM, systemic mastocytosis; MC, mast cell.

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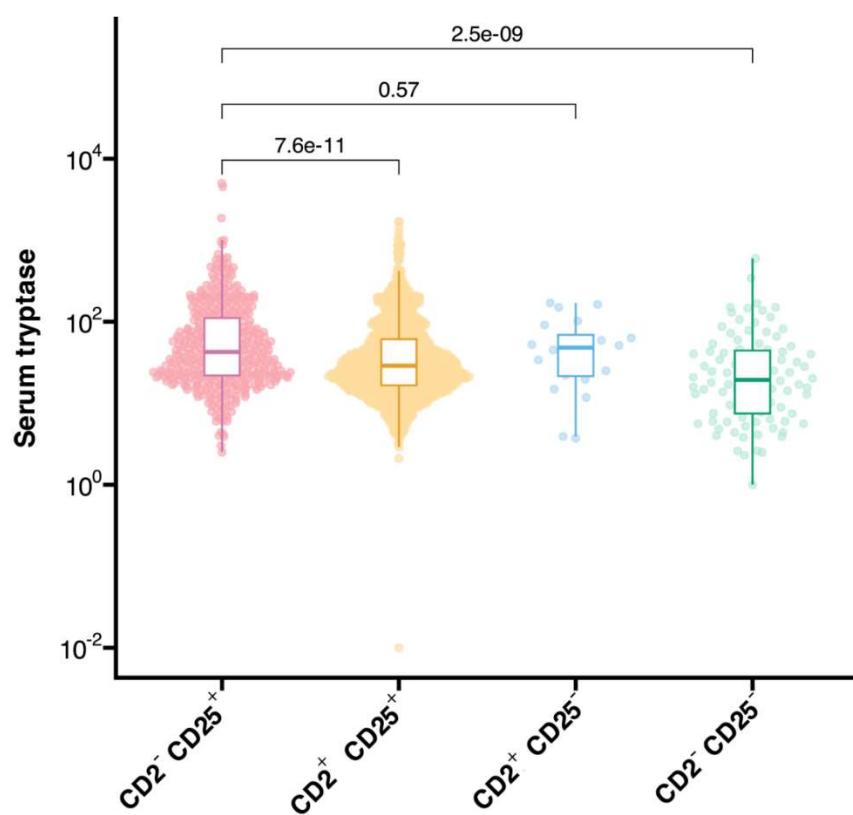
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706 Supplemental Figure S15

707 Serum tryptase level in patients with SM stratified by CD2 and CD25 expression patterns in MC

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711 Four groups of patients with SM and serum tryptase level at diagnosis were examined based on the
712 pattern of CD2 and CD25 expression on/in MC as determined by flow cytometry or immunohisto-
713 chemistry: CD2-/CD25⁺ MC, CD2⁺/CD25⁺ MC, CD2⁺/CD25⁻ MC; and CD2-/CD25⁻ MC. Patients with
714 lack of CD2 expression in MC had a significantly higher median serum tryptase level compared to
715 patients in whom MC were found to express CD2.

716 Abbreviations: SM, systemic mastocytosis; MC, mast cell.

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