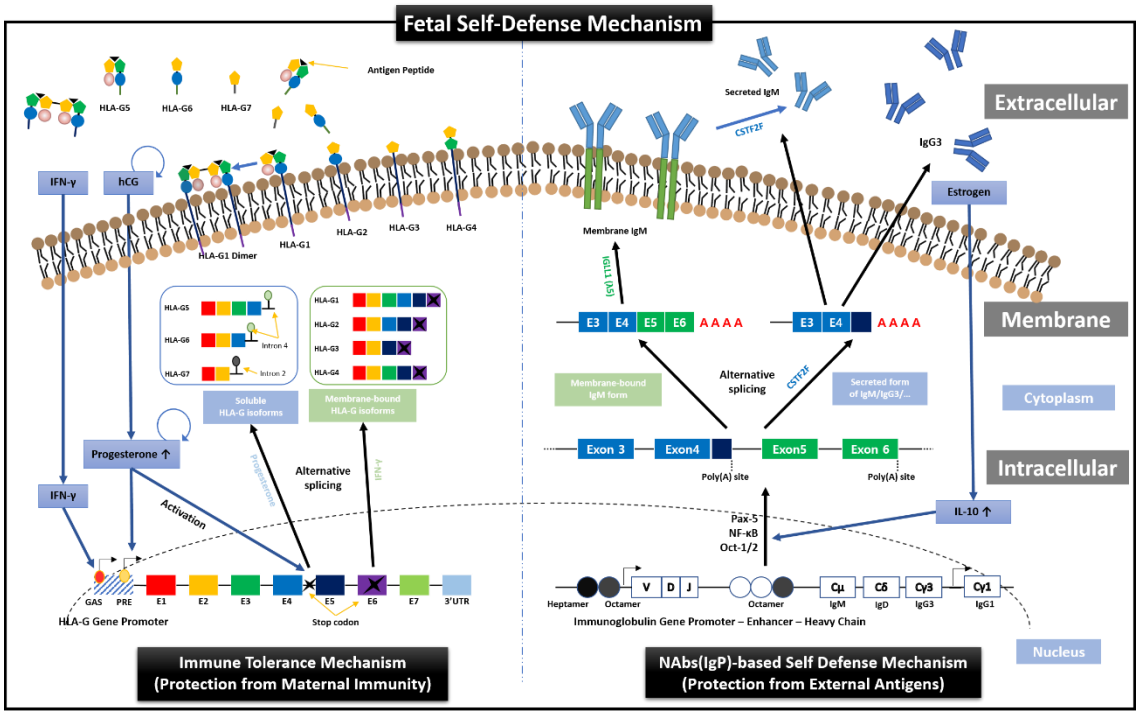
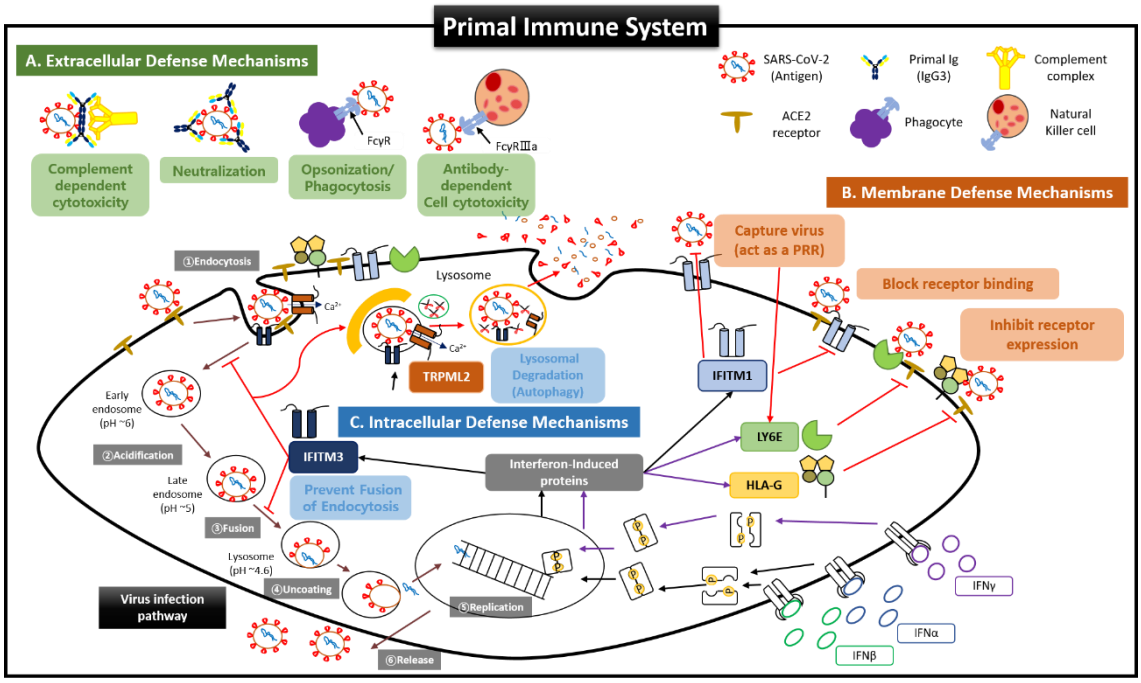
Supplemental Information



Supplemental Figure 1. Overview of HLA-G-based immune tolerance environment and NAbs-based self-defense mechanism induced by hormones secreted during pregnancy

During pregnancy, the fetus must protect itself from various foreign pathogens and the maternal immune system. Protection from the maternal immune system is achieved by an immune tolerance environment induced by various HLA-G isoforms secreted mainly by EVTs, which continuously infiltrate the maternal decidua. The transcription, alternative splicing, and secretion of soluble HLA-G isoforms, which can induce extensive immune tolerance, are induced by the pregnancy-related hormones hCG and progesterone (28), and the transcription and expression of membrane-bound HLA-G, which induces local immune tolerance, is also upregulated by interferon stimulation (the left panel). On the other hand, we suggest that before the immune system establishment, the fetal self-defense mechanism against foreign pathogens can be induced by various factors, including IL-10, Pax-5, NF-κB, and Oct-1/2, that promote the transcription of Igs, especially NAbs, such as IgG3 and IgM, which have the most outstanding anti-viral effector

functions, through the pathway triggered by estrogen, another pregnancy-related hormone secreted from trophoblasts (the right panel). Therefore, our results demonstrate that hormones secreted during pregnancy may induce the different immune systems that fully protect the fetus from maternal immune system and foreign antigens.



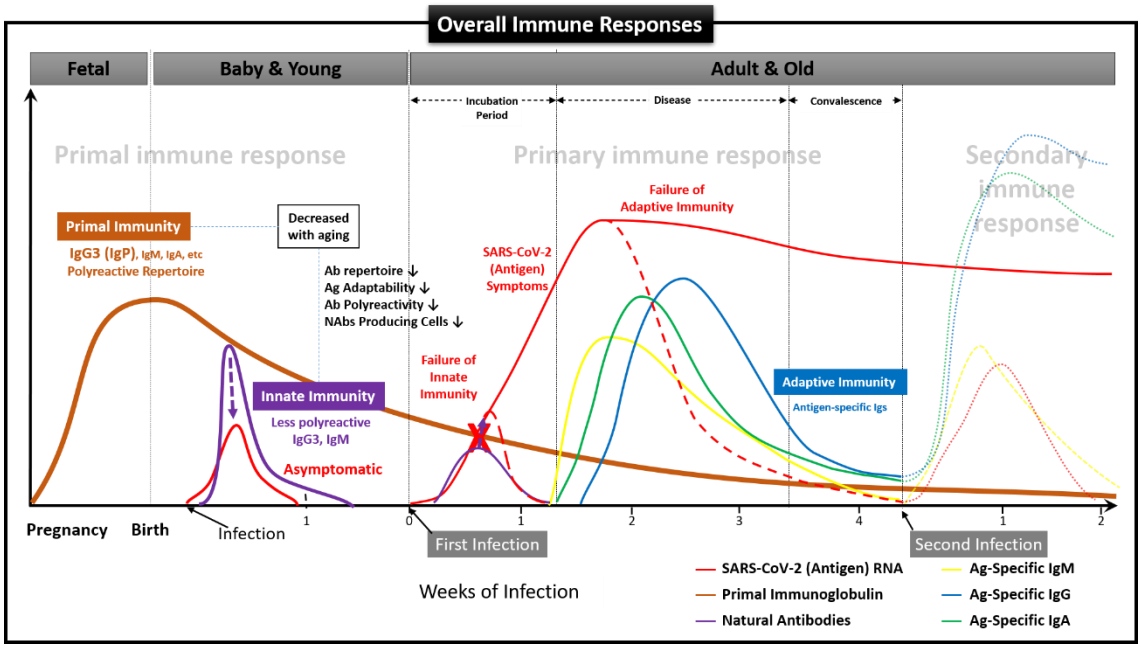
Supplemental Figure 2. Suggested Primal Immune System as a Self Defense Mechanism of Fetal Stem Cells before the establishment of innate and adaptive immune systems

Since virus invasion and infection into host cells are affected by the host's innate and adaptive immune responses, it was recognized that the fetus was very susceptible to viral infection before forming innate and adaptive immune systems. However, our results for fetal stem cells in early pregnancy suggest that the fetus possesses very complicated and sophisticated self-defense mechanisms (primal immune system) at the cellular level exist in addition to the protective function of the placental trophoblast cells, which was considered the only barrier to protect the fetus from external infections so far.

(A) Extracellular defense mechanism by secretion of NAbs and complement proteins. Our results show that fetal stem cells in early pregnancy can produce and secrete various NAbs, including IgP (fetal IgG3) polyreactive to unexperienced antigens, along with complement proteins to eliminate external pathogens immediately by CDC and ADCC, before umbilical cord generation, the main delivery route of maternal IgG, and before the complete development of B cells. In particular, IgP secreted from early pregnancy fetal stem cells was proved to have the primal Ig

repertoire that can instantly recognize various antigenic epitopes pathogens. Furthermore, IgP has the most outstanding Fc effector function to induce ADCC by binding with the highest affinity to Fcγ receptor of NK cells, which accounts for most of the fetal immune cells. These functions of IgP may contribute to much effective removal of the infectious agents. Also, we demonstrated that itPG-FSCs could secrete various NAbs such as IgP, IgM, and IgA as they contained factors, including Igll1, Pax5, and Cstf, that promote the secretion of soluble Igs. This finding may indicate that the primal immune system can establish the protective mechanism against various infection routes such as the upper respiratory tract in addition to the placenta and maternal blood. (B) The defense mechanism in the cellular and endosomal membranes to inhibit virus entry and replication by interferon-inducing proteins. Over the past decade, several interferon-inducible cellular proteins, including interferon-inducible transmembrane family (IFITMs), ArfGAP with dual pleckstrin homology (PH) domains 2 (ADAP2), gamma-interferon-inducible lysosome/endosome-localized thiolreductase (GILT), and Lymphocyte antigen 6 family member E (LY6E), have been known to regulate the infectious entry of various viruses (39). Still, few studies have been performed on the fetus during pregnancy. However, we proved through a new ex-vivo culture condition containing HSC/UCB-MSC^{CO}-EVs that the expressions of interferon-inducible proteins such as IFITM3, LY6E, and HLA-G were increased in fetal stem cells by increased interferon stimulation. Our results that itPG-FSC-EVs contained a higher level of LY6E and IFITM3 than FSC-EVs suggest that fetal stem cells also have a sophisticated self-protection mechanism that suppresses the viral infection by LY6E mainly expressed in the plasma membrane at the beginning of virus entry and by IFITM3 expressed in the endosomal membrane after cell entry. In addition, membrane-bound HLA-G, whose expression is increased by interferon stimulation, may inhibit the relative expression of receptors that can be used as entry pathways of viruses and protect infected host cells by inducing delayed immune response through trogocytosis by contact with immune cells (47).

(C) Intercellular defense mechanism to inhibit virus transmission by activating anti-viral autophagy. Transient Receptor Potential Mucolipin Subfamily (TRPMLs) are proteins constituting endosome cation channels and perform various physiological functions. TRPML1 is receiving attention as a target molecule that inhibits the fusion of the SARS-CoV-2 envelopes and endosomes (62), and a novel role of TRPML2, only expressed in the recycled endosome, in the innate immune response was recently revealed (41), but there is still little study on fetal stem cells during pregnancy. From our results, the expression of TRPML2 was increased in itPG-FSC EVs. We intend to suggest a new mechanism that the viral antigen information degraded by IFITMs upregulated by interferon stimulation acts as a pattern recognition receptor (PRR), thereby increasing TRPML2 expression (39, 41). In addition, upregulated TRPML2 may contribute to the intracellular protective mechanism that can prevent the replication of infected inhibits virus by binding to the endosomes containing the virus entering the cell, thereby inhibiting the entry of the virus into the cell nucleus and by inducing the degradation through anti-viral autophagy (lysosomal degradation).



Supplemental Figure 3. Suggested Immune System for the whole life

To date, the antibody-based humoral immune system against foreign pathogen infection is divided into three categories according to the infection stage (20). The first is the immediate innate immune response by NAbs already present in the body before antigen exposure, and it acts as the first line of defense against infectious agents. The second is an extrafollicular "innate-like" antibody response induced within the early few days after antigen exposure, which serves to eliminate the infection temporarily until the specialized antibody response matures. Lastly, it is an acquired antibody response that appears delayed 1-2 weeks after infection by highly assembled antibodies through somatic cell hypermutation and class switch recombination processes.

However, we found that the primal immune system by IgP existed before the fetal immune system establishment during pregnancy, and the adaptabilities of innate and adaptive immune systems against foreign antigens depend on the primal repertoire that was experienced and formed during this period.

(A) Primal Immunity. According to the study for healthy fetuses in early pregnancy (57), circulating B lymphocytes in fetal blood at 12 weeks of gestation have a diverse BCR repertoire,

and IGHV-containing clones analyzed at 26 weeks of gestation are similar to those of healthy infants. It was confirmed that it exists in proportion, but this can be called the primary repertoire. On the contrary, the repertoire of natural antibodies, including IgG3 secreted from fetal stem cells obtained around 10 weeks of pregnancy, which we confirmed through this study, has not experienced any antigens except for themselves, and in other words, can respond to any antigens. Therefore, it can be a primal repertoire, and it will be gradually diversified to the primary repertoires with antigen-specific diversity due to modifying factors such as antibodies transmitted from the mother, food and environmental factors, vaccinations, infections, or diseases (57).

(B) Innate Immunity. The primary repertoire formed from the Primal repertoire in early pregnancy plays a critical role in manifesting symptoms after infection in the later innate and adaptive immune systems. Younger children who have a relatively similar repertoire to the Primal repertoire can react immediately to new antigens and eliminate them before the adaptive immune system acts. However, since birth, the number of naïve B-1 cells that secrete natural antibodies acting on the innate immune system, the ability to secrete antibodies, and the adaptability of the repertoire to new antigens gradually decrease with age. The ability to cope with new viruses, such as SARS-CoV-2, also decreases with age (59). For this reason, many children and younger generations have asymptomatic infections of COVID-19, and even young children are well adapted to the mutant virus (63), while the older, the more symptomatic and severe the proportion of patients increases.

(C) Adaptive Immunity. The evasion mechanisms to the initial innate immunity of all viruses, including SARS-CoV-2, and the resulting delayed priming of T cell responses and adaptive immunity are receiving attention as the main factors that increase the severity and fatality of COVID-19 with age (64). In other words, as the naïve T cell pool gradually decreases with age, it is difficult to generate a T cell response capable of recognizing a new antigen. This assumption can be supported by the findings that systemic excessive immune pathology, including cytokine

storms, is caused by an explosive innate immune response activated to replace the delayed adaptive immune system (6). In particular, the delayed adaptive immune response can cause taste and olfactory abnormalities in asymptomatic and mild patients accounting for more than half of SARS-CoV-2 infections (65), so the most effective prevention and treatment for various infections including SARS-CoV-2 It can be said that the strategy lies in an effective innate immune system capable of triggering a normal adaptive immune response. From this point of view, it is expected that our research results, which first proposed the concept of the primordial immune system before the formation of the innate immune system, will contribute to finding a fundamental solution to protect humanity against unknown infectious agents in the future. Another important finding in our results is that IgA is also contained in itPG-FSC-EVs secreted from FSCs in early pregnancy. It can be expected to provide the expanded variety of applicability to various infection pathways such as the upper respiratory tract in the existing IgG-centered antibody therapeutics.