Text S3. Effects of endogenous pMHC

We modify the ODE model presented in the main text to investigate the effects of a large concentration of identical pMHC molecules. In this calculation, we assume the contact interface to be a flat disc (radius 5 μm) containing a pMHC concentration of $M = 500 \, \mu m^{-2}$ and a TCR concentration of $R = 100 \, \mu m^{-2}$. We first reformulate the model from the perspective of a single TCR interacting with a homogeneous pMHC distribution. Next, we use this modified model to calculate the probability of productive signaling for a single TCR and assuming TCR do not compete for pMHC (i.e. $M \gg R$), we calculate the probability that at least 1 TCR (out of 7854) at the T cell-APC contact interface has transduced a productive signal from interacting with 39270 pMHC.

In this model, we introduce additional states, denoted as $\hat{U}_j$, that track the probability of finding a TCR without pMHC (i.e. once it diffuses away) in an intermediate state. In this state, a different pMHC may bind TCR and resume signaling from where a previous pMHC left off (provided $\mu$ is sufficiently small). The ODE system describing this modified model is,

$$
\begin{align*}
\frac{\partial B_0}{\partial t} &= -k_{on}B_0 + k_{off}B_0^c + k_{on}U_0 - (k_{off} + k_p)B_0 \\
\frac{\partial B_0^c}{\partial t} &= k_{on}B_0 - k_{off}B_0^c + k_{on}U_0^c - (k_{off} + k_p)B_0^c \\
\frac{\partial U_0}{\partial t} &= -k_{on}U_0 + k_{off}U_0^c - (k_- + k_{on})U_0 + k_+M\hat{U}_0 + k_{off}B_0 + \mu \sum_{j=1}^{S-1} U_j \\
\frac{\partial U_0^c}{\partial t} &= k_{on}U_0^c - k_{off}U_0^c - (k_- + k_{on})U_0 + k_+M\hat{U}_0^c + \mu \sum_{j=1}^{S-1} U_j \\
\frac{\partial \hat{U}_0}{\partial t} &= k_- U_0 - k_+M\hat{U}_0 + \mu \sum_{j=1}^{S-1} \hat{U}_j \\
\frac{\partial B_j}{\partial t} &= -k_{on}B_j + k_{off}B_j^c + k_pB_{j-1} - (k_p + k_{off})B_j + k_{on}U_j \\
\frac{\partial B_j^c}{\partial t} &= k_{on}B_j - k_{off}B_j^c + k_pB_{j-1}^c - (k_p + k_{off})B_j^c + k_{on}U_j^c \\
\frac{\partial U_j}{\partial t} &= -k_{on}U_j + k_{off}U_j^c - (k_- + k_{on} + \mu)U_j + k_+M\hat{U}_j + k_{off}B_j \\
\frac{\partial U_j^c}{\partial t} &= k_{on}U_j^c - k_{off}U_j^c - (k_- + k_{on} + \mu)U_j^c + k_{off}B_j^c \\
\frac{\partial \hat{U}_j}{\partial t} &= k_- U_j - k_+M\hat{U}_j - \mu \hat{U}_j \\
\frac{\partial B_{S-1}}{\partial t} &= k_pB_{S-1} \\
\frac{\partial B_{S-1}^c}{\partial t} &= k_pB_{S-1}^c
\end{align*}
$$

where $M$ is the pMHC concentration and all other parameters are defined in the main text. The probability of productive signaling through a single TCR is $B_S + B_S^c$ and the probability that at least 1 TCR at the T cell-APC contact interface has transduced a productive signal is simply $1 - (1 - B_S - B_S^c)^{7854}$. We plot this quantity in Figure 5 in the main text. As an example, consider an endogenous pMHC with $k_{off} = 5 \, s^{-1}$ and $k_{on} = 0.001 \, \mu m^{-2}$. When $\mu = 100 \, s^{-1}$ this pMHC does not produce a productive signal (Figure 5C) despite forming $\sim 100,000$ TCR/pMHC bonds during 30 s ($= 1/(1/k_{off} + 1/(k_{on}[pMHC])))(7853$ TCR)(30s) = 107, 100).