Natural Killer (NK) cells are educated during development to distinguish self as human leukocyte antigen (HLA) using inhibitory killer-cell immunoglobulin-like receptors (KIR). NK cells are educated for response if they express a KIR for which a corresponding HLA ligand is present, whereas NK cells expressing KIR for which there is no corresponding HLA ligand are rendered hypo-functional in comparison. This educational process is termed licensing. While it is clear that licensed and unlicensed NK cells are functionally distinct, it is unclear how this process occurs. Opposing models of “arming,” “disarming,” and “tuning” have been proposed.

To address this gap in knowledge, licensed and unlicensed human NK cells were sorted and then expanded on the Clone9.mbIL21 K562 expansion platform. Unlicensed NK cells expanded significantly less over 3 weeks than licensed NK cells (896.5 vs. 47,929). Using the sorted and expanded licensed or unlicensed NK cells, activating (NKp46) or inhibitory (KIR) receptors were cross-linked to induce receptor signaling followed by reverse-phase protein array analysis. Phospho-AMPK-α, the catalytic subunit of AMPK and phospho-p38α were both upregulated in licensed NK cells compared to unlicensed NK cells. Using the AMPK agonist, Metformin, or the AMPK inhibitor, Compound C, the role of AMPK in licensed or unlicensed NK cells was determined. Inhibiting AMPK blocked the expansion and cytotoxicity of both NK cell subsets. Lastly, using teloFISH mass cytometry, licensed and unlicensed NK cell telomere lengths were determined. As expected, telomere lengths of immature (CD56brCD16neg) NK cells were much longer than mature (CD56dimCD16pos) NK cells. Unlicensed NK cells had a statistically non-significant trend toward increased telomere lengths compared to licensed NK cells. The metabolic pathway activation and the trend in telomere lengths are most consistent with the “arming” model of NK cell education. Additional data is needed to determine whether the difference in telomere lengths is significant.