Meeting with Iain (February 11) - StarCellBio Signal Transduction Assignment

- Randomization of the proteins in question.

- What is happening during the process: can you create a model for what is happening here.

- Epistasis analysis --> order the pathway

* Changes in localization
  + extracellular ligand and signal transduction: plasma membrane, nuclear localization
    - plasma membrane that does not change
    - cytoplasm --> nucleus
    - cytoplasm --> plasma membrane
    - cytoplasm that does not change
  + - and + ligand
  + - and + inhibitor (should be intermediate in the pathway) - downstream of the one in the middle
  + levels do not change = phosphospecfiic antibodies that come from protein phospho-specific antibody This site is known to be phosphorylated by protein wide analysis that works against a specific phosphorylation site from a proteomic screen)
* Does not care about the in-vitro data
* Phosphorylation = becomes phosphorylated by ligand addition
  + the plasma membrane that stays and the one that goes to the nucleus for example
  + 2 of them should get phosphorylated one of them should not, two downstream ones are inhibited by the inhibitor
  + one is always phosphorylated one is never phosphorylated

1. What happens to each protein: phosphorylated, change in localization.

* Is the phosphorylation site specific for this pathway?
* Does the inhibitor treatment affect the phosphorylation of this site.

2. Pathway: define the pathway and at which point the inhibitor is acting.

3. Explain the reasoning by which you order the pathway

Controls:

* GFP alone = cytoplasmic + nuclear
* Nuclear = Histone H2B
* Cytoplasm = GFP fused to something large without a signal sequence goes to cytoplasm (100kDa)
* Plasma membrane = transmembrane domain protein with an internal signal sequence fused to GFP