**Results 2: Phenotypes for shRNA expressing RAW264.7**

- shRNA-mediated RNAi: multiple phenotypes for C5a and IgG2a, fewer for UDP.

### Genes that were knocked-down

<table>
<thead>
<tr>
<th>Gene</th>
<th>Control</th>
<th>200x</th>
<th>500x</th>
<th>1000x</th>
</tr>
</thead>
<tbody>
<tr>
<td>C5aR</td>
<td>1.00</td>
<td>0.01</td>
<td>0.02</td>
<td>0.03</td>
</tr>
<tr>
<td>IgG2a</td>
<td>1.00</td>
<td>0.01</td>
<td>0.02</td>
<td>0.03</td>
</tr>
</tbody>
</table>

### Gene expression

<table>
<thead>
<tr>
<th>Gene</th>
<th>200x</th>
<th>500x</th>
<th>1000x</th>
</tr>
</thead>
<tbody>
<tr>
<td>C5aR</td>
<td>1.00</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>IgG2a</td>
<td>1.00</td>
<td>0.01</td>
<td>0.02</td>
</tr>
</tbody>
</table>

### Conclusion and summary:

- RNAi can be efficiently used in RAW264.7 cells
- Using prescreened shRNA sequences, target knock-down was ≥80% in almost all transduced lines, ≥90% in two-thirds, and ≥99% in one-third.
- 37 Lines have been studied, covering 31 targets (more in the pipeline, at rate of 4/week).
- Apart from receptor knock-downs, alterations in signaling were observed in:
  - One-third of responses to C5a
  - Only one response to UDP (more suppressed statistically)
  - Expected phenotypes are usually (but not always) detected.
  - Unexpected phenotypes were frequent.
- Results could be replicated.

### Approaches to validation of RNAi phenotypes:

- Replicate lines with different shRNAs
- Alternate KD strategy (antisense, siRNA)
- Microarrays to look for off-target effects
- Knockdown reversal
- Test (and confirm) a likely hypothesis (e.g., with a 2nd knockdown)