

Supplement Materials

Figure S1. 96 well ELISA plates were coated with Stat3 β -BSA peptide or Stat3 β free peptide for O/N. The plate was washed, blocked with BSA in PBS, washed and appropriate control and test antisera from immunized mice #146–150 (A–E) were added and incubated overnight at 4 °C. The plates were then washed, incubated with secondary goat anti-mouse IgG (Fc specific)-HRP for 2.5 h. After another round of wash, the plates were developed, using OPD as substrate, and OD was read at 490 nM.

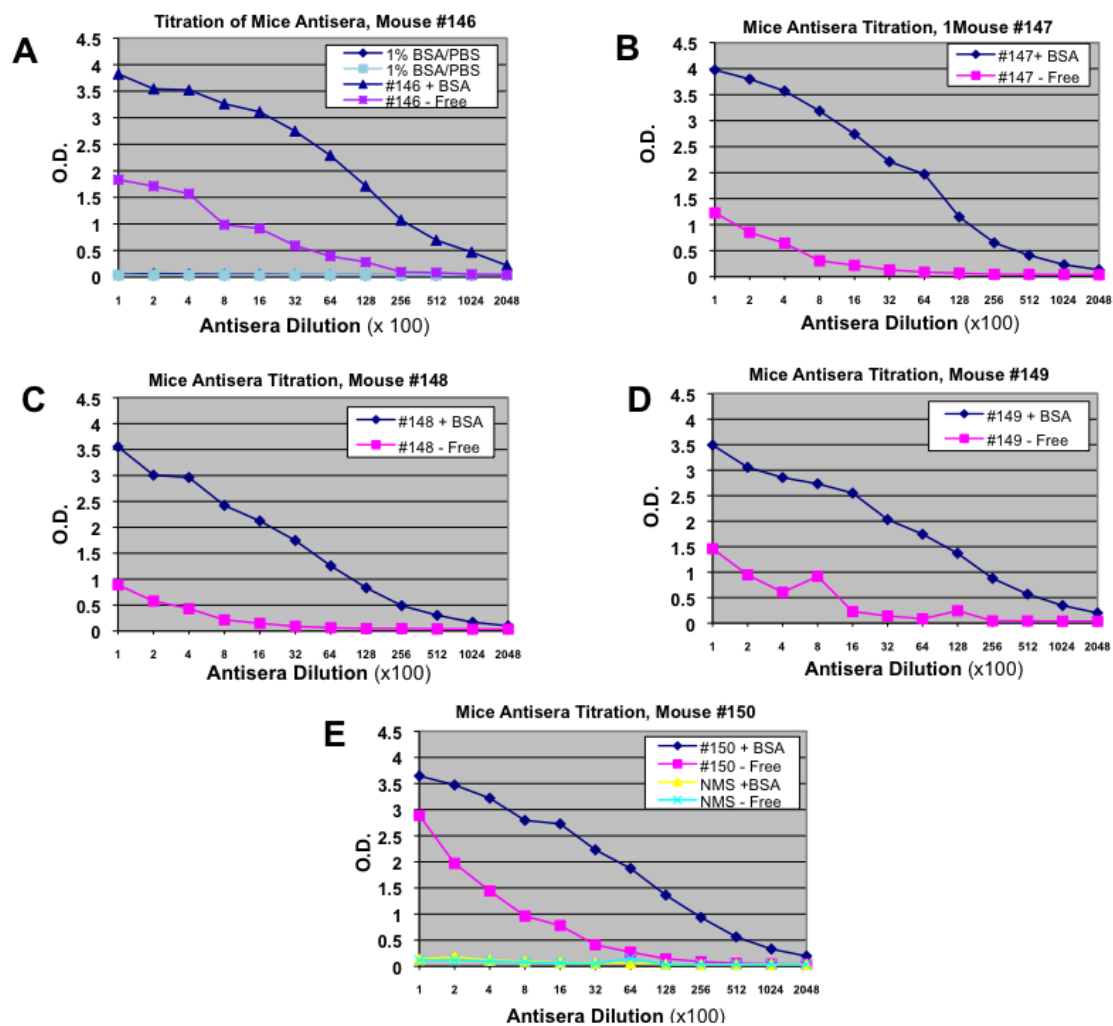


Figure S2. Screening of 6 hybridoma supernatants cloned from mouse #147 by immunoblotting, using lysates from 293 T cells transiently transfected with Stat3- α -GFP, Stat3- β -GFP.

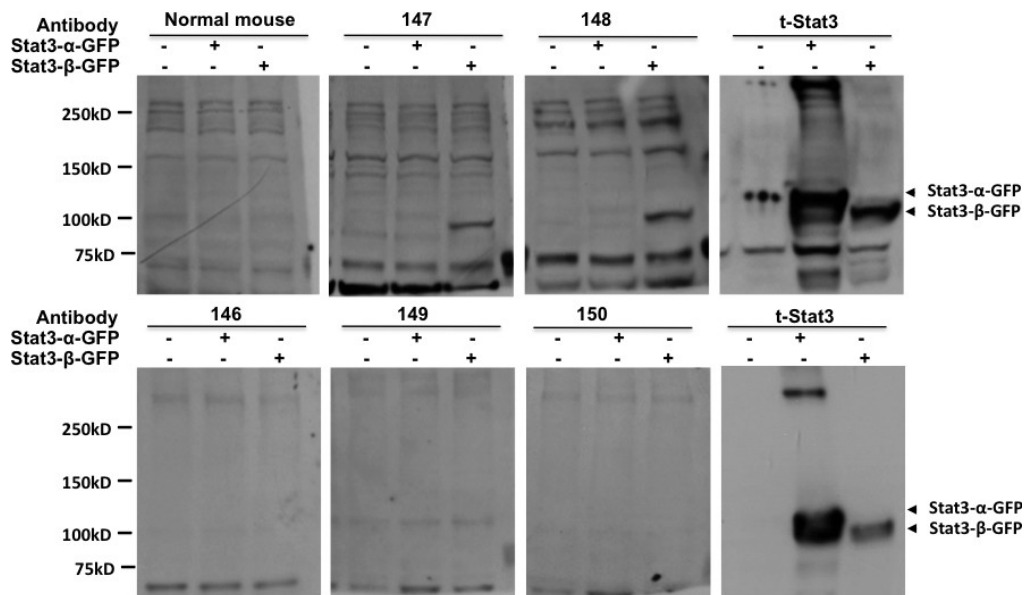


Figure S3. Screening of 6 hybridoma supernatants cloned from mouse #147 by immunoblotting using lysates from 293 T cells transiently transfected with Stat3- α -GFP, Stat3- β -GFP.

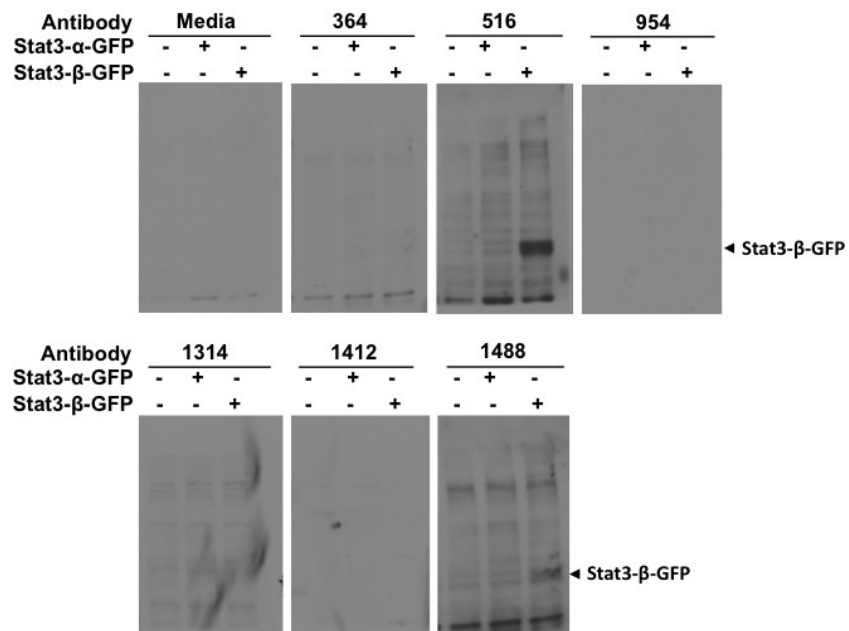


Figure S4. Screening of sups from sub clones of hybridoma clones (A) 516, and (B) 954, and 1488 by immunoblotting, using lysates from 293 T cells transiently transfected with Stat3- α -GFP, Stat3- β -GFP.

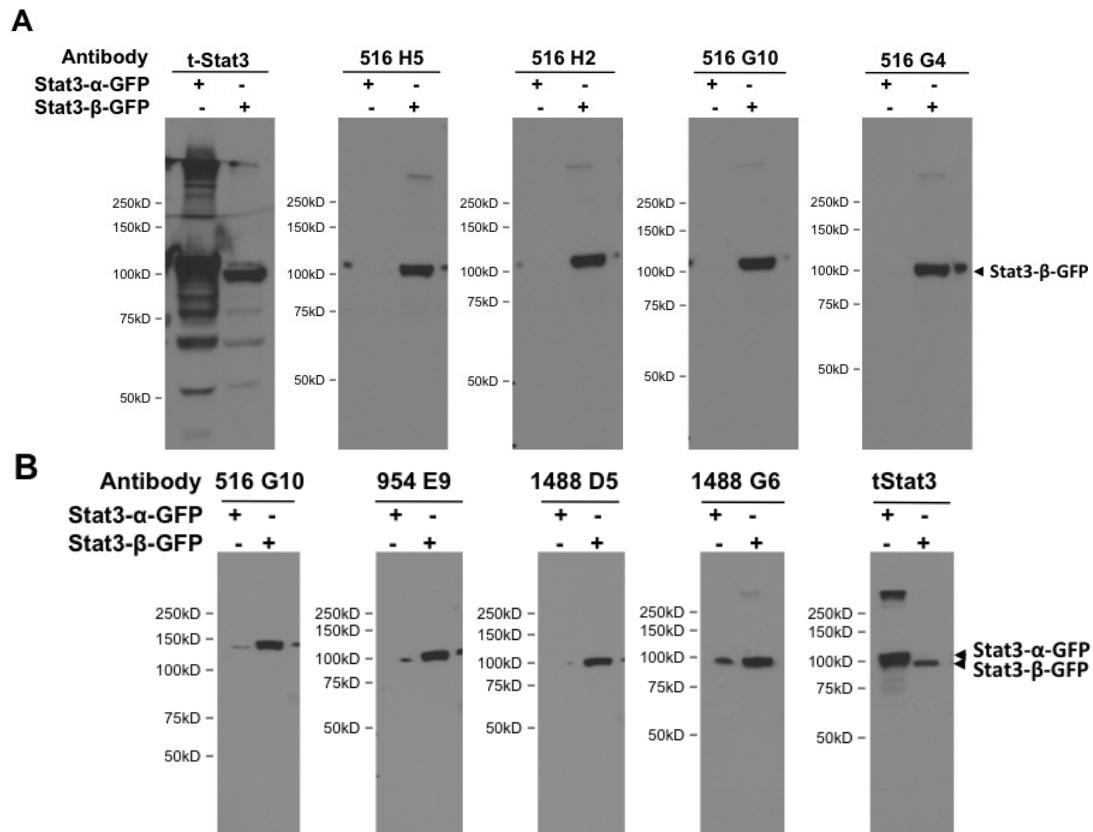


Figure S5. Screening of sups from 4 sub clones of hybridoma clones 516G10, 954E9, 1488G6 by immunoblotting, using lysates from 293 T cells transiently transfected with Stat3- α -GFP, Stat3- β -GFP.

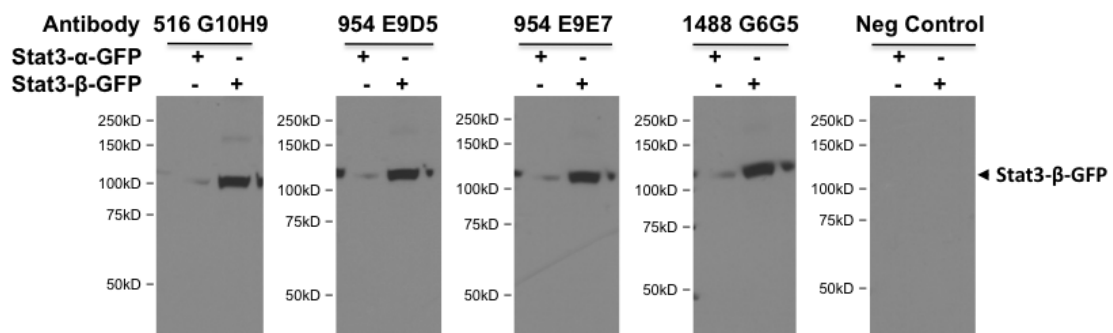


Figure S6. Decreasing amounts (100 ng, 30 ng, 10 ng, 3 ng, 1 ng, 0.3 ng) of truncated Stat3 β (amino acids 127–722) and truncated Stat3 β devoid of CT7 (amino acids 127–717) were loaded onto consecutive wells and subjected to SDS-PAGE, transferred to nitrocellulose membranes and probed with the three monoclonal antibodies 516 G10H9, 954 E9E7 and 1488 G6G5 as well as total Stat3 antibody (clone 124H6) and visualized by chemiluminescence as described in Figure 4. The X-ray film was either exposed to the chemiluminescence for either 10 s (A) or 2 min (B).

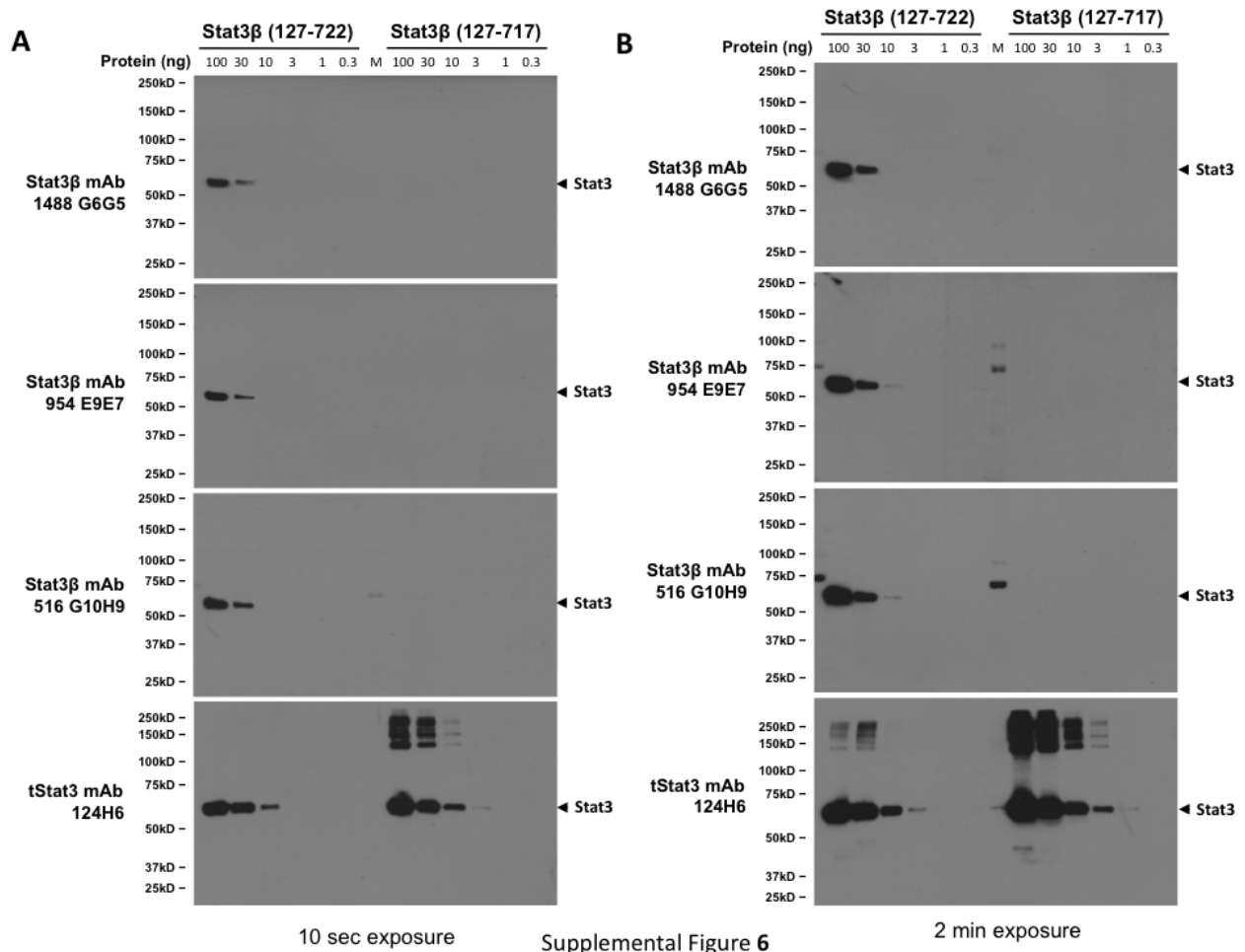


Table S1. Elisa data showing reactivity of anti-sera against Stat3- β immunizing peptide.

| Dilution | 1% BSA/PBS | 1% BSA/PBS | #146 + BSA | #146 – Free | #147 + BSA | #147 – Free | #148 + BSA | #148 – Free | #149 + BSA | #149 – Free | #150 + BSA | #150 – Free | NMS + BSA | NMS – Free |
|-----------------|-----------------------|-----------------------|-----------------------|------------------------|-----------------------|------------------------|-----------------------|------------------------|-----------------------|------------------------|-----------------------|------------------------|----------------------|-----------------------|
| 100 | 0.048 | 0.037 | 3.824 | 1.833 | 3.976 | 1.228 | 3.552 | 0.889 | 3.492 | 1.46 | 3.645 | 2.889 | 0.132 | 0.114 |
| 200 | 0.063 | 0.038 | 3.544 | 1.711 | 3.798 | 0.85 | 3.006 | 0.578 | 3.056 | 0.947 | 3.473 | 1.971 | 0.181 | 0.114 |
| 400 | 0.057 | 0.038 | 3.523 | 1.569 | 3.57 | 0.643 | 2.96 | 0.432 | 2.855 | 0.605 | 3.219 | 1.442 | 0.113 | 0.099 |
| 800 | 0.049 | 0.038 | 3.263 | 0.985 | 3.185 | 0.304 | 2.421 | 0.216 | 2.733 | 0.918 | 2.794 | 0.963 | 0.094 | 0.078 |
| 1600 | 0.05 | 0.036 | 3.111 | 0.913 | 2.741 | 0.219 | 2.122 | 0.147 | 2.551 | 0.228 | 2.728 | 0.78 | 0.072 | 0.064 |
| 3200 | 0.04 | 0.036 | 2.749 | 0.591 | 2.211 | 0.131 | 1.746 | 0.09 | 2.031 | 0.14 | 2.23 | 0.411 | 0.057 | 0.051 |
| 6400 | 0.039 | 0.037 | 2.29 | 0.392 | 1.97 | 0.087 | 1.256 | 0.063 | 1.744 | 0.085 | 1.874 | 0.271 | 0.061 | 0.146 |
| 12800 | 0.039 | 0.037 | 1.715 | 0.282 | 1.147 | 0.066 | 0.833 | 0.049 | 1.369 | 0.242 | 1.363 | 0.144 | 0.04 | 0.033 |
| 25600 | 0.037 | 0.037 | 1.073 | 0.095 | 0.651 | 0.043 | 0.489 | 0.048 | 0.877 | 0.044 | 0.935 | 0.088 | 0.037 | 0.038 |
| 51200 | 0.038 | 0.037 | 0.693 | 0.082 | 0.411 | 0.042 | 0.304 | 0.039 | 0.568 | 0.043 | 0.563 | 0.056 | 0.037 | 0.037 |
| 102400 | 0.037 | 0.036 | 0.467 | 0.051 | 0.229 | 0.039 | 0.169 | 0.035 | 0.345 | 0.039 | 0.329 | 0.044 | 0.035 | 0.037 |
| 204800 | 0.036 | 0.037 | 0.217 | 0.043 | 0.133 | 0.036 | 0.1 | 0.037 | 0.199 | 0.038 | 0.195 | 0.04 | 0.035 | 0.037 |

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