

82

83

Supplementary Figure 4

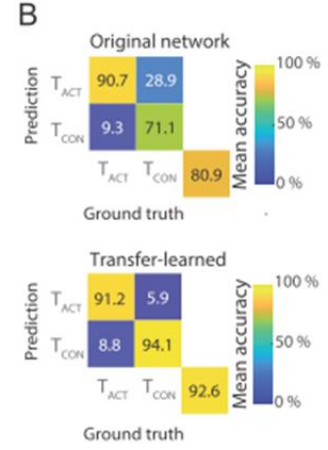
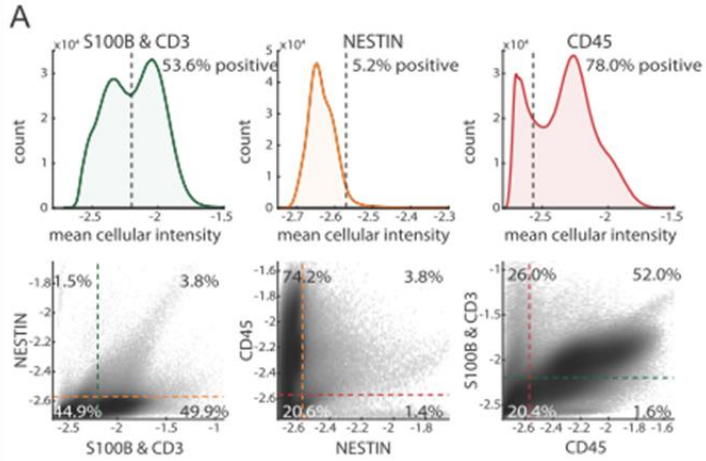
84

85

86

87

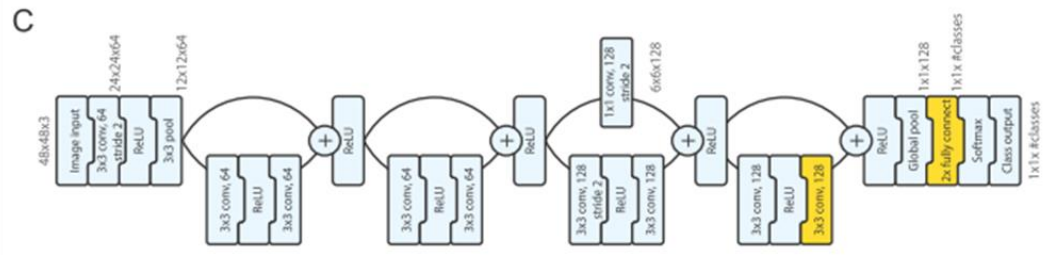
88



89

90

91



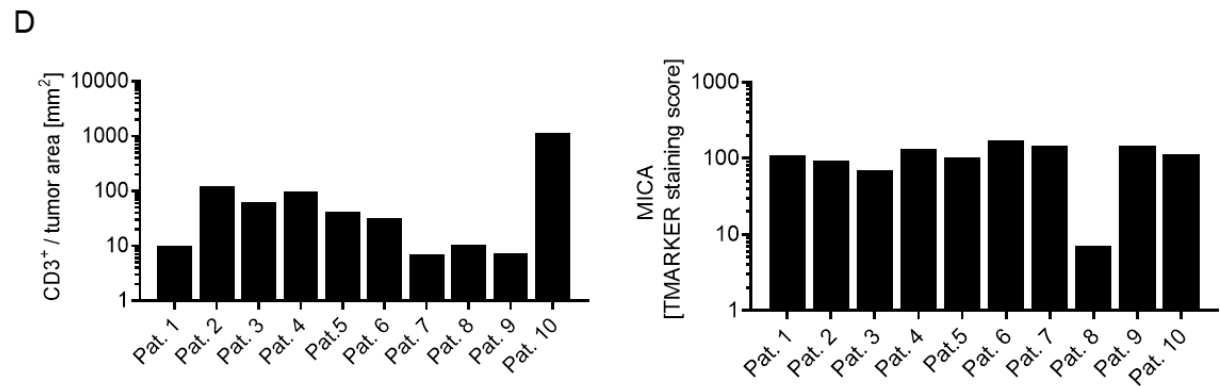
92

93

94

95

96



97

98

99

100

101

102

103 **Supplementary Figure 4. Image-based analyses of co-cultures of cells from patient-derived**
104 **glioblastoma samples with mRNA-modified T cells** **A.** Thresholding strategy for marker-based
105 classification of cells. Histograms and scatter plots of single-cell marker intensity distributions
106 for S100B & CD3 (green, 488), NESTIN (yellow, PE), CD45 (red, APC). Data represents the
107 entire set of cells imaged and analyzed. Single linear thresholds were set for each cellular marker
108 as indicated. **B.** Convolutional neural network (CNN) test dataset accuracy. Accuracy of the
109 original network (top) and the transfer-learned network on the manually curated test dataset of
110 T_{ACT} and T_{CON} cells (bottom). The transfer-learned network (TNet) was subsequently used in all
111 morphological analyses of T cells presented in this paper. **C.** Architecture of the original CNN
112 from. Layers, where weights and biases were reset prior to the retraining of the transfer network,
113 are highlighted in yellow. **D.** Patient tumors that were used for the image-based platform were
114 stained for CD3⁺ T cells and the NKG2D ligands MICA and ULBP2. Staining intensity for each
115 tumor is shown.