SUPPLEMENTARY FIGURE LEGENDS

Supplementary Fig. S1

Activated T cells and tumor infiltrating lymphocytes express PD-1, B7-H1 (PD-L1), CD137 and OX40. (A) Flow cytometry analyses of the immunostaining with the indicated antibodies on CD8⁺ and CD4⁺ gated OT-1 and OT-2 splenocytes activated for 48h with the respective cognate antigens as synthetic peptides. Histograms depicting expression on resting T cells are overlaid for reference as indicated in the legend. (B) Flow cytometry analyses of gated CD8⁺ and CD4⁺ T lymphocytes from individual livers harvested from c-myc OVA tg+ mice at week 3 of age. Results show both the percentage of positive cells and the MFI upon immunostaining for PD-1, B7-H1, CD137 and OX40. Data are representative of three independent experiments.

Supplementary Fig. S2

Three week old mice present established multifocal hepatocellular carcinomas and neonatal mice express OVA mRNA in their livers. (A) Representative photomicrographs (H&E stained section, 200x and 400x magnification) demonstrating multifocal hepatocellular carcinoma in livers of c-myc OVA tg+ mice (left) induced perinatally and sacrificed at three weeks of age but not in WT mice bred under identical conditions (right). (B) RT-PCR analysis of expression of mRNA encoding OVA in the livers of HCC hosting 8 week od transgenic mice, and 5 days old puppies whose mothers had been off doxycycline since day +1. Lanes indicated with (+) show a reaction including retrotranscriptase (RT), while parallel lanes with samples without
retrotranscriptase are indicated with (-). Perinatally expressed OVA antigen in
the liver determines more propensity to give rise to tolerance.

Supplementary Fig. S3

Anti-CTLA-4 mAb treatment fails to extend survival of c-myc OVA tg+
mice. Parallel experiments to those in Fig. 2 showing the lack of effect on
survival in mice treated with two doses of 100 μg of anti-CTLA-4 mAb (clone
9D9) on days 21 and 25 or the addition of these doses to Combo3 (Combo4
group). Fraction of surviving mice per group on day 250 is provided in the
legend. P values refer to rat IgG treated control group analyzed by log rank test.
Ns, non significant; *, P < 0.05; ***, P < 0.001.

Supplementary Fig. S4

Combo3 treatment induces infiltrates of blastic T lymphocytes and
increases the CD8/Treg ratio. (A) Experiments as in Fig. 4C and D showing
the absolute numbers of retrieved CD8⁺ and CD4⁺ lymphocytes for the indicated
treatment groups and the number of blastic cells gated as shown in the
corresponding dot plots. (B) Experiments as in Fig. 4A depicting the absolute
numbers of Foxp3⁺ CD4⁺ lymphocytes and the ratio of the absolute numbers of
CD8⁺ lymphocytes to Foxp3⁺ CD4⁺ regulatory T cells. #, absolute number of
cells. *, P < 0.05; **, P < 0.01.
Supplementary Fig. S5.

**Granzyme B, perforin and FasL are upregulated on CD8+ T cells after Combo3 treatment.** Flow cytometry analysis of intracellular immunofluorescence for granzyme B and perforin and surface expression of FasL (CD95L) and TRAIL on immunomagnetically isolated CD8+ liver TILS of mice treated with control antibody or the indicated double or triple combinations on days 21 and 25. Mice were sacrificed for analysis on day 29 as in figure 4B. Representative individual histograms are presented and the percentage±SD of positive lymphocytes (blue) and MFI±SD (red) are included in each histogram representing each group of mice.

Supplementary Fig. S6

**HCC cell lines derived from c-myc OVA tg+ mice express OVA and functional antigen presenting molecules.** (A) Phase contrast images of cultures of repeatedly passaged cell lines from HCCs harvested from terminally ill c-myc OVA tg+ mice. (B) Immunofluorescence and FACS analysis for the expression of the EpCAM epithelial marker and B7-H1, H-2K^b^, H-2D^b^ and H-2IA^b^. Shaded histograms represent isotype-matched background staining, open histograms baseline expression, and black histograms expression following culture with recombinant IFNγ for 48h. (C) RT-PCR analyses with primers for the indicated genes in the JMJ cell lines. Liver tissue from WT and c-myc OVA tg+ mice, EL-4 cell line, EL-4 OVA transfectant EG-7 and mouse liver cancer Hepa 1.6 cells are included as controls. Lanes indicated with (+) show a
reaction including retrotranscriptase (RT), while parallel lanes with samples without retrotranscriptase are indicated with (-). (D) Specific cytotoxicity in 5h-chromium release assays of activated OT-1 lymphocytes at the indicated effector:target ratios on JMJ9 cells (precultured or not with IFNγ for 48h), EL-4 or OVA⁺ EL-4 cells (EG-7). This experiment was repeated twice with similar results.

**Supplementary Fig. S7**

**TILs recognize unknown antigens in JMJ cell lines.** Experiments performed coculturing immunoselected CD8⁺ TILs (A) or splenocytes and irradiated JMJ7, JMJ9, MC38 or MC38-OVA cells. IFNγ concentration in the cocultures supernatants were assessed by ELISA. PMA+ionomycin (Iono) was used as a positive control and T cells without any cell line (-) were used as a negative control.