Exons 26, 27, and 34 of Notch 1 were amplified by PCR from genomic DNA from thymi, spleen and bone marrow using primers described elsewhere (O'Neil J, Calvo J, McKenna K et al. Activating Notch1 mutations in mouse models of T-ALL. Blood. 2006;107(2):781–785). The products were sequenced and results analyzed using a CLC genomics workbench (Aarhaus, Denmark).

Table S1. Notch mutations present in tumors derived from LMO2-2A-γc overexpression on an Arf−/− background

<table>
<thead>
<tr>
<th>Mouse number</th>
<th>Genotype</th>
<th>Nucleotide change</th>
<th>Amino acid change</th>
</tr>
</thead>
<tbody>
<tr>
<td>507</td>
<td>γc</td>
<td>WT</td>
<td></td>
</tr>
<tr>
<td>515</td>
<td>γc</td>
<td>WT</td>
<td></td>
</tr>
<tr>
<td>516</td>
<td>LMO2-2A-γc</td>
<td>Multiple nucleotides added in the pest domain</td>
<td>Premature stop in Pest domain</td>
</tr>
<tr>
<td>517</td>
<td>LMO2-2A-γc</td>
<td>T inserted at pos 7296</td>
<td>Premature stop in Pest domain</td>
</tr>
<tr>
<td>518</td>
<td>LMO2-2A-γc</td>
<td>T-G at pos 7230</td>
<td>Serine-Arginine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G-A at pos 7444</td>
<td>Glutamic acid-lysine</td>
</tr>
<tr>
<td>519</td>
<td>LMO2-2A-γc</td>
<td>T-G at pos 7230</td>
<td>Serine-Arginine</td>
</tr>
<tr>
<td>520</td>
<td>LMO2-2A-γc</td>
<td></td>
<td></td>
</tr>
<tr>
<td>521</td>
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<td>G-A at pos 7444</td>
<td>Glutamic acid-lysine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C-T at pos 7518</td>
<td>Serine-phenylalanine</td>
</tr>
<tr>
<td>523</td>
<td>LMO2-2A-γc</td>
<td>C-T at pos 7518</td>
<td>Serine-phenylalanine</td>
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<td>526</td>
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<td>G-A at pos 7444</td>
<td>Glutamic acid-lysine</td>
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**Figure S1. Phenotype of preleukmic and tumor populations**
GFP expression in the spleen and thymus of a pre-leukemic Lmo2-GFP, Arf<sup>−/−</sup> mouse at day 88 post transplant. The phenotypes of T and B cell tumors that developed in the cohort of mice receiving Lmo2-GFP, Arf<sup>−/−</sup> cells is also shown. Note the lower degree of GFP expression in the CD4<sup>+</sup>, CD8<sup>+</sup> T cell tumor.

**Figure S2. Lmo2 impedes thymocyte maturation at the DN2 stage**
Antibody-dependent microbead-mediated depletion was used to remove the double (CD4<sup>+</sup>CD8<sup>+</sup>) and single (CD4<sup>+</sup> and CD8<sup>+</sup>) positive cells from the thymus of three Arf<sup>+/+</sup> and three Arf<sup>−/−</sup> animals. The remaining double negative thymocytes were transduced with either MSCV-IRES-mCherry or MSCV-Lmo2-IRES-mCherry. Transduced thymocytes were cultured in vitro on OP9-DL1 stromal cells together with IL-7 and FLT-3. After 14 days, transduced thymocytes expressing mCherry for mature (CD4 and CD8) and immature (CD44 and CD25) T cell markers, sorted to obtain the DN2 population (CD4<sup>+</sup>CD25<sup>+</sup>), and replated at the same density on OP9-DL1 stromal cells. Seven days later, mature T cells (CD4<sup>+</sup>,CD8<sup>+</sup> and single positive) are absent in the Lmo2, Arf<sup>+/+</sup> and the Lmo2, Arf<sup>−/−</sup> populations.

**Figure S3. Schematic illustrating the experimental design for Fig. 3A**
Thymi from Arf<sup>+/+</sup> and Arf<sup>−/−</sup> animals were removed and enriched for immature (CD4<sup>−</sup>CD8<sup>−</sup>) thymocytes using a magnetic column. These immature thymocytes were transduced with Lmo2 by co-culture with MSCV-Lmo2-mCherry vector producing cells. These transduced thymocytes were moved to OP9-DL1 cells and were cultured in vitro together with the cytokines IL-7 and FLT-3. Every 3–5 days the thymocytes were plated onto fresh OP-DL1 cells and after 14 days an aliquot of the thymocytes was analyzed for development into mature T cells. At day 20 the thymocytes were sorted for mCherry<sup>+</sup>DN2 cells and 2 × 10<sup>5</sup> of either Arf<sup>−/−</sup> or Arf<sup>+/+</sup> mCherry<sup>+</sup>DN2 cells together with 2 × 10<sup>5</sup> WT bone marrow cells were transplanted into lethally irradiated WT recipients.

**Figure S4. The Arf promoter is engaged in Lmo2<sup>+</sup> thymocytes at the DN2 stage**
CD4<sup>−</sup>/CD8<sup>−</sup> double-negative Arf<sub>Gfp/Gfp</sub> thymocytes transduced with MSCV-Lmo2-IRES-mCherry were cultured in vitro on OP9-DL1 stromal cells together with IL-7 and FLT-3. After 20 days in culture, thymocytes were sorted to recover mCherry-positive cells at the DN2 stage. 2 × 10<sup>5</sup> DN2 mCherry<sup>+</sup> thymocytes were transplanted into lethally-irradiated mice together with 2 × 10<sup>5</sup> bone marrow cells. The levels of GFP and mCherry in a thymus from a mouse six weeks post transplant is shown in the top panel. Gating on three populations (mCherry and GFP positive, mCherry positive and mCherry negative) demonstrates that there is a high grade DN2 block present in Lmo2<sup>+</sup> thymocytes that have Arf promoter engagement.

**Figure S5. Characterization of tumors arising in Arf<sup>Cre</sup>; Rosa26<sup>Lsl-Notch</sup> mice**
(A) Comparison of GFP expression in individual tissues revealed no statistically significant differences between Arf<sup>Cre</sup>; Rosa26<sup>Lsl-Notch</sup> (n = 4) and Arf<sup>Cre−</sup>; Rosa26<sup>Lsl-Notch</sup> (n = 17) mice. (B) Arf<sup>Cre</sup>; Rosa26<sup>Lsl-Notch</sup> tumors are transplantable. 1 × 10<sup>7</sup> lymph node cells from a moribund Arf<sup>Cre−</sup>; Rosa26<sup>Lsl-Notch</sup> donor mouse were injected by tail vein into 5 healthy, non-irradiated C57Bl/6 recipient mice, all of which succumbed to overt clinical disease recapitulating that of the donor. The percentages of cells expressing Notch-IRES-GFP in the hematopoietic tissues of the donor (open bars) and recipients (grey bars) were determined by flow cytometry. (C)
Activation of the \( \text{ROSA}^{\text{LSL-Notch}} \) allele in pre-leukemic mice. Flow cytometry revealed low levels of GFP expression in the hematologic tissues of four \( \text{Arf}^{\text{Cre/}}; \text{Rosa26}^{\text{LSL-Notch}} \) mice killed at day of life 308 with no signs of clinical disease.
Figure S1

Pre-leukemic

- Spleen
- Thymus

T cell lymphoma

- Spleen
- Thymus

B cell lymphoma

- Spleen
- Thymus

Peripheral blood

Legend:

- Spleen
- Thymus
- Bone Marrow
- Peripheral blood

CD4 and CD8 percentages in each tissue are indicated, along with GFP expression levels.
Figure S2

- mCherry, Arf^{+/+}
- LMO2, Arf^{+/+}
- mCherry, Arf^{-/-}
- LMO2, Arf^{-/-}
1) Thymi removed from Arf+/+ and Arf−/− mice

2) Enrich for the CD4-CD8- thymocytes using a magnetic column

3) Co-culture of CD4-CD8-thymocytes with MSCV-Lmo2-mCherry virus producer cells

4) CD4-CD8- cells co-cultured with OP9-DL1 cells

6) Analyze for development into mature T cells

7) mCherry positive DN2 thymocytes are sterile sorted

8) Transplantation of:
   - 2x10^5 Lmo2, Arf+/+
   - 2x10^5 Lmo2, Arf−/−
   - 2x10^5 WT bone marrow cells into lethally irradiated WT recipient mice

Day 14

Day 20
Figure S4

Lmo2, Arf^{Gfp/Gfp} Thymus

mCherry and GFP Positive

mCherry Negative

GFP

mCherry

CD4

CD8

CD44

CD25

CD4

CD8

6 weeks post thymocyte transplant
Figure S5