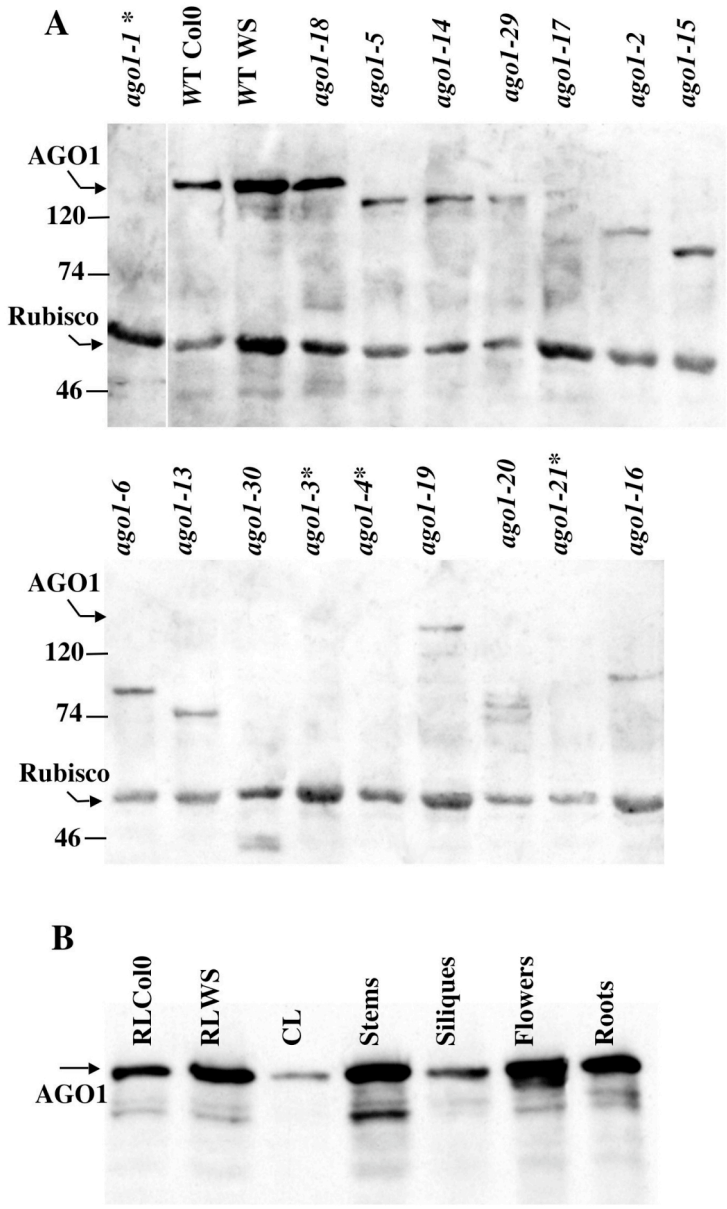
Supplemental Figure 1: Western blots of Argonaute proteins

In order to check whether these strong alleles were null alleles, we verified the presence of the AGO1 protein in 17 EMS or T-DNA alleles (SFig1A). The protein was absent in only four of them (*ago1-1*, *ago1-3*, *ago1-4*, *ago1-21*). In all the others, except for *ago1-18,* a truncated protein of variable size could be detected. The *ago1-18* allele still showed an AGO1 protein of WT size. These results indicate that not all the mutants showing a strong phenotype can be considered as null allele mutants. We confirm here the ubiquitous expression of this gene, as the protein is indeed present in all the organs, throughout the development of the plant (SFig. 1B).

(A) Western blot with protein extracts from the different alleles. Stars indicate the null alleles.
(B) Western blot with protein extracts from different organs of WT. Proteins from rosette leaves (RL) were extracted from both Col0 and WS. The other organs were from Col



Purification of antibodies

The anti-ago serum was kindly provided by C. Benning (Michigan State University, USA). Antibodies were raised in rabbits against N-terminal 6xHis-tagged fusion protein expressed in *E. coli* strain M15 (Qiagen). The immunizing antigen encompasses amino acids 145-318 of the AGO1 protein (Benning personnal communication). The serum was subjected to affinity purification as follows: A glutathione S-transferase (GST-) fusion protein of AGO1 (amino acids 1-434) prepared in pGEX2T (Pharmacia) and was expressed in *E. coli* BL21/pLysS (Clontech) and purified by standard methods (Smith and Johnson 1988). In addition crude bacterial extracts from *E. coli* BL21/pLysS were prepared. Purified GST-AGO1 (GST crude extracts) in 100 mM MOPS, pH 8.0 were coupled to Affigel-10 beads (BioRad) following the manufacturer's instruction. The remaining active coupling sites were blocked with 100 mM Tris, pH 8, before subjecting the beads to one round of the washing/elution regimen (see below) prior to use. Diluted serum (in PBS) was first passed over a column containing GST/extract-coupled Affigel matrix, and then over the GST-AGO affinity matrix. Bound antibodies were washed with PBS + 500 mM NaCl, then with 3 column volumes elution buffer (0.5 M NH_4OH, 3 M KSCN, 1 mg/ml BSA) (Earnshaw and Rothfield 1985, *Chromosoma* 91: 313-321), and immediately subjected to gel filtration against PBS with Sephadex G-25 spin columns prior to concentration on Centricon-30 (Amicon) molecular sieves.

Preparation of plant protein extracts

The different alleles of *ago1* were grown *in vitro* for 3 weeks. Seedlings were regularly transferred on fresh medium once a week. Entire seedlings were then frozen in liquid nitrogen. Frozen plant material was stored at –80°C before protein extraction. For comparison between aerial organs, *Arabidopsis thaliana* ecotype Col0 was grown in the greenhouse. Rosette leaves, stems, cauline leaves and flowers were collected and immediately frozen in liquid nitrogen. Roots were collected from Col0 seedlings grown *in vitro* for 3 weeks. Seedlings were regularly transferred on fresh medium once a week.

Liquid nitrogen-frozen plant tissue (0.5 g) ground to a fine powder was homogenized in 2 ml guanidine-HCL buffer (6M Guanidine-HCL, 10 mM Tris-HCL, 100 mM NaCl, pH 8.0), briefly sonicated and clarified by centrifugation. Such extracts were either stored at -20°C for later use, or appropriate aliquots were centrifuged to remove remaining plant material, and precipitated in ice-cold 5% trichloroacetic acid (TCA). Following centrifugation, precipitates were washed once in ice-cold 100% ethanol before boiling in Laemmli protein loading dye.

SDS-PAGE and western blot

Proteins were separated by SDS-PAGE on 10% acrylamide (37.5:1 acryl:bis) gels at 180V following the protocol discribed in Sambrook et al. (1989, *Molecular cloning : a laboratory manual*, Cold Spring Harbor, New-York). Gels were either stained with Coomassie blue, to estimate protein quantity, or transferred to Hybond C membrane using a transblot SD semidry transfer cell (BioRad), following the manufacturer's instructions, except that the minimum time to transfer was 1 hour. Protein blots, prepared by electrotransfer onto Hybond C+ membranes (Amersham), were briefly stained with Ponceau-S to reveal the prominent RubisCo band, which served as a useful loading control (not shown). Purified antibodies were used at a dilution of 1:500 (with respect to the initial starting volume of crude serum), and immunoblots were revealed with reagents and protocols of the western Star kit (Cetus-Perkin-Elmer).